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UNIVERSITY OF ALBERTA

Synthesis of Diaminopimelic Acid (DAP) and Analogs; Inhibition Studies on DAP Dehydrogenase and DAP Epimerase

by

© Patricia Lane-Bell

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Chemistry

EDMONTON, ALBERTA

Fall 1991

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Synthesis of Diaminopimelic Acid (DAP) and Analogs; Inhibition Studies on DAP Dehydrogenase and DAP Epimerase submitted by Patricia Lane-Bell in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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To Stephen and my mother

Abstract

Two nitrogen-modified analogs of diaminopimelic acid (DAP), *N*-hydroxy DAP (**3**) and *N*-amino DAP (**4**), were synthesized as mixtures of all possible isomers for testing against diaminopimelate dehydrogenase and diaminopimelate epimerase. Potential transition-state analogs γ-glutamyl hydrazide (**6**) and *N'*-methyl-γ-glutamyl hydrazide (**7**) were prepared in stereochemically pure form and also tested against these enzymes. Compounds **6** and **7** were found to be neither substrates nor inhibitors for both DAP epimerase and DAP dehydrogenase. Analogs **3** and **4** were found to be substrates for DAP dehydrogenase. Tests against DAP epimerase revealed that **4** was a modest competitive inhibitor (K_i = 2.9 mM) but **3** was an extremely potent competitive inhibitor (K_i = 0.0056 mM).

A stereocontrolled synthesis of DAP was achieved which employed a Lewis-acid-catalyzed ene reaction between *trans*-2-phenylcyclohexyl glyoxylates **89** and **90** and protected L-allylglycine **69** as the key step. The homoallylic alcohols **91** and **92** produced were subsequently transformed into the selectively protected DAP derivatives **117** and **118** and then to DAPs **1a** and **1b** in ratios of 14:86 (L,L: *meso*) and 84:16 (L,L: *meso*), respectively. The potential expansion of this method to other analogs and other selectively protected DAPs was demonstrated by the preparation of **4**, and the nitrogen-protected diacids **119** and **120**. A simple NMR method for the determination of DAP stereochemistry using the bis-camphanamide dimethyl esters of DAP was also developed. This method was also successfully used with α-aminopimelates **14**, **15** and **133**.

Phosphonate analogs **8-13** and amino acids **14-18** were examined as potential substrates and inhibitors of DAP dehydrogenase and DAP epimerase. The phosphonate analogs **8-12** were found to weakly inhibit DAP dehydrogenase, precluding the use of a coupled enzyme assay for DAP epimerase. The known radiochemical assay for DAP epimerase was expanded to provide inhibition constants for the analogs tested. Phosphonate analogs **8**,**9** and **11** were weak competitive inhibitors, and with the exception

of the 4-fluoro DAP analog **18**, the remaining amino acids examined showed weak or no inhibition of this enzyme. Based on these results the apparent active site requirements for the epimerase were discussed.

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I wish to thank my supervisor, Dr. John C. Vederas, for his guidance during my studies, and I am grateful to Dr. Fionna Martin and Dr. Mark Zabriskie for proof-reading this manuscript. Help of postdoctoral fellows both past and present for assistance in running deuterium and other NMR spectra is also acknowledged, especially that of Dr. Paul Reese, Dr. Bernard Rawlings, Dr. Greg Helms, Dr. Evon Bolessa and Dr. Mark Zabriskie. The following people are gratefully acknowledged for their collaborative work in the enzyme studies: Dr. Michael A. Pickard, Dr. Lister Lam, Dr. Monica Palcic and Ms. Suzanne Crawley. Thanks are extended to the staff of spectral and analytical services for their assistance in characterizing compounds, and to E. Jason Wasylyk for his assistance as a summer research student.

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List of Abbreviations

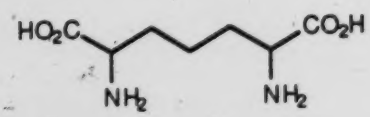
A	absorbance units
Ac	acetyl
AcOH	acetic acid
ADP	adenosine 5'-diphosphate
Ala	alanine
Anal.	analysis
Ar	aryl
ATP	adenosine 5'-triphosphate
AZIDAP	2-(4-amino-4-carboxybutyl)-2-aziridine-carboxylic acid
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
%C	% of bis-acrylamide in the total weight of acrylamide
calcd	calculated
CI	chemical ionization
DAP	diaminopimelic acid
DCC	dicyclohexylcarbodiimide
DEAE	diethylaminoethyl
DMAP	4-*N*,*N*-dimethylaminopyridine
DMAP·HCl	4-*N*,*N*-dimethylaminopyridine hydrochloride
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DSS	sodium 2,2-dimethyl-2-silapentane-5-sulfonate
DTT	dithiothreitol
e.e.	enantiomeric excess

EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
FPLC	fast protein liquid chromatography
g	gram
GC	gas-liquid chromatography
GlcNAc	*N*-acetylglucosamine
Glu	glutamic acid
HPLC	high pressure liquid chromatography
iPrOH	isopropyl alcohol
IR	infrared spectroscopy
K_i	competitive inhibition constant
K_i'	noncompetitive inhibition constant
K_m	Michaelis-Menten constant in absence of inhibitor
$K_m^{(I)}$	Michaelis-Menten constant in presence of inhibitor
L	liter
LDA	lithium diisopropyl amide
MCPBA	*meta*-chloroperbenzoic acid
MeOH	methanol
MS	mass spectrometry
Ms	methanesulfonyl
MsCl	methanesulfonyl chloride
MTPA	2-methoxy-2-(trifluoromethyl)phenylacetyl
MTPACl	2-methoxy-2-(trifluoromethyl)phenylacetyl chloride
MurNAc	*N*-acetylmuramic acid
MW	molecular weight
$NADP^+$	nicotinamide adenine dinucleotide phosphate

NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
n-BuLi	n-butyllithium
NMR	nuclear magnetic resonance
Ph	phenyl
PLAP	pig liver acetone powder
PLE	pig liver esterase
PLP	pyridoxal phosphate
PMSF	phenylmethylsulfonyl fluoride
Pos FAB	positive fast atom bombardment
psi	pounds per square inch
Stds	standards
%T	total percentage of acrylamide, sum of acrylamide monomer and crosslinker, bis-acrylamide
THF	tetrahydrofuran
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TMS	tetramethylsilane
Tris	tris(hydroxymethyl)aminomethane
U	unit
Z	carbobenzyloxy
Z-Cl	benzyl chloroformate

Introduction

Diaminopimelic acid (DAP) (**1**), discovered and isolated almost 40 years ago from *Corynebacterium diphtheria*,[1] and the intermediates and enzymes in its biosynthetic pathway have recently provided interesting and challenging targets for the development of new antibiotics. This symmetrical amino acid is an important constituent in the peptidoglycan of most bacteria[2-4] and is also a key intermediate in the biosynthesis of lysine.[5]



1

Peptidoglycan, a net -like polymer which is the main structural component in the cell wall of Gram negative and Gram positive bacteria, is responsible for maintaining the integrity and shape of the bacterium and protecting the cell from lysis under ordinary hypotonic conditions in its environment.[3, 4] This polymer consists of linear glycan strands of alternating β-1,4 linked *N*-acetyl glucosamine (GlcNAc) and its 3-*O*-D-lactyl ether, *N*-acetylmuramic acid (MurNAc). The MurNAc residues are substituted on the D-lactyl group by pentapeptide units generally having the composition L-alanine (or glycine), γ-D-glutamate, a diamino acid such as lysine or DAP, followed by two D-alanines[2-4] (Figure 1). To give peptidoglycan its necessary insolubility and rigidity, the glycan strands are crosslinked by peptide bridges. These bridges may involve the direct formation of a peptide bond between the penultimate D-alanine of one peptide with the free ω-amino group of the diamino acid in an adjacent glycan chain, or it may involve an additional intervening amino acid residue or peptide (Figure 2). The composition of these peptides and the manner in which they crosslink can vary greatly and this has been used as a method of classifying bacteria.[3, 6] In Gram-negative bacteria the crosslinks are most often

the direct type. Those pentapeptides which do not participate in crosslinking eventually lose either one or both of the D-alanine residues by the action of carboxypeptidases.[2]

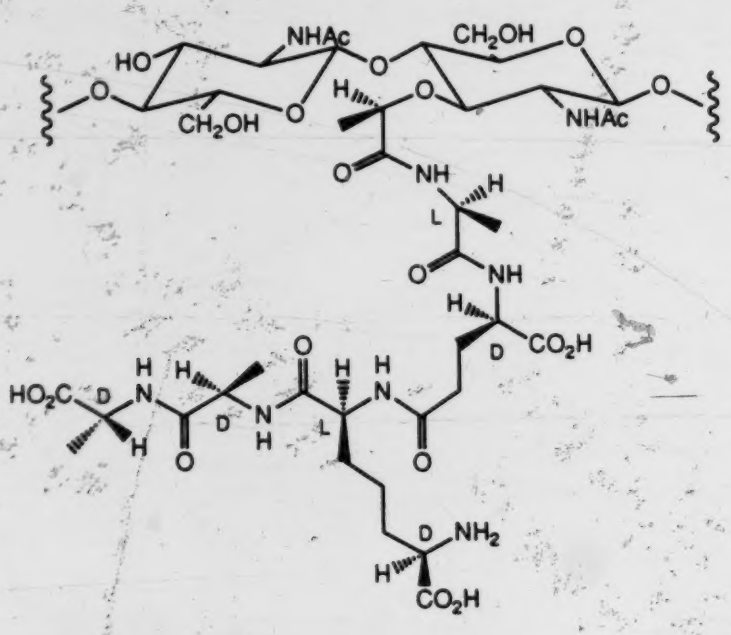
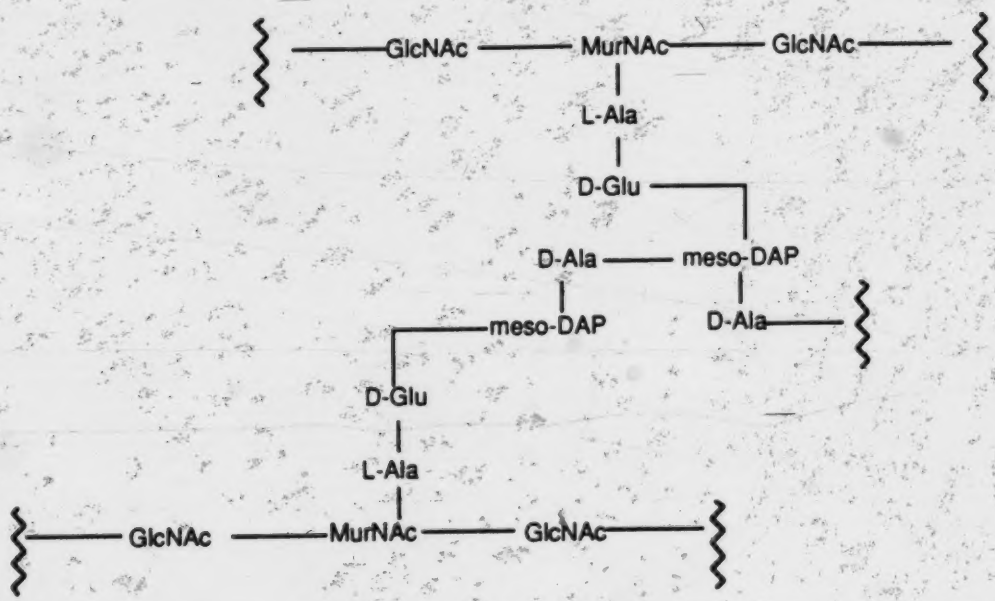


Figure 1. *E. coli* Peptidoglycan Monomer

Although *meso*-DAP is the most common isomer found in the peptidoglycan of Gram-negative bacteria and bacilli, the occurrence of D,D-DAP,[7-11] L,L-DAP,[3, 12] and DAP analogs such as 3-hydroxy-DAP[13, 14] and lanthionine[15] has also been reported. Evidence for more complex and novel crossbridges involving two or more DAP residues has also been discovered.[3, 16] DAP may also serve to anchor lipoproteins to the peptidoglycan by amide bond formation between the distal carboxyl moiety of DAP and the ω-amino group of a lysine residue in the lipoprotein.[17, 18]

Figure 2. Crosslinking of Peptidoglycan in *E. coli*



DAP is also an important intermediate in the biosynthesis of lysine in bacteria[5, 19] and in higher plants[20-30] (Figure 3). Lysine is synthesized from aspartate which, through the action of the enzymes aspartate kinase,[5, 31] aspartate semialdehyde dehydrogenase, dihydrodipicolinate synthase,[32] and dihydrodipicolinate reductase,[33, 34] forms tetrahydrodipicolinate. At this stage, depending on the type of bacteria, one of two pathways may be followed. In most bacteria, tetrahydrodipicolinate is converted to L,L-*N*-succinyl-DAP by tetrahydrodipicolinate succinylase[35, 36] and succinyl DAP aminotranferase.[37] Desuccinylation by succinyl DAP desuccinylase[38, 39] affords L,L-DAP. Epimerization to *meso* -DAP by diaminopimelate epimerase[40, 41] and subsequent decarboxylation by diaminopimelate decarboxylase[42, 43] produces L-lysine. In other bacteria, tetrahydrodipicolinate is converted directly to *meso*-DAP by the action of diaminopimelate dehydrogenase.[44-49]

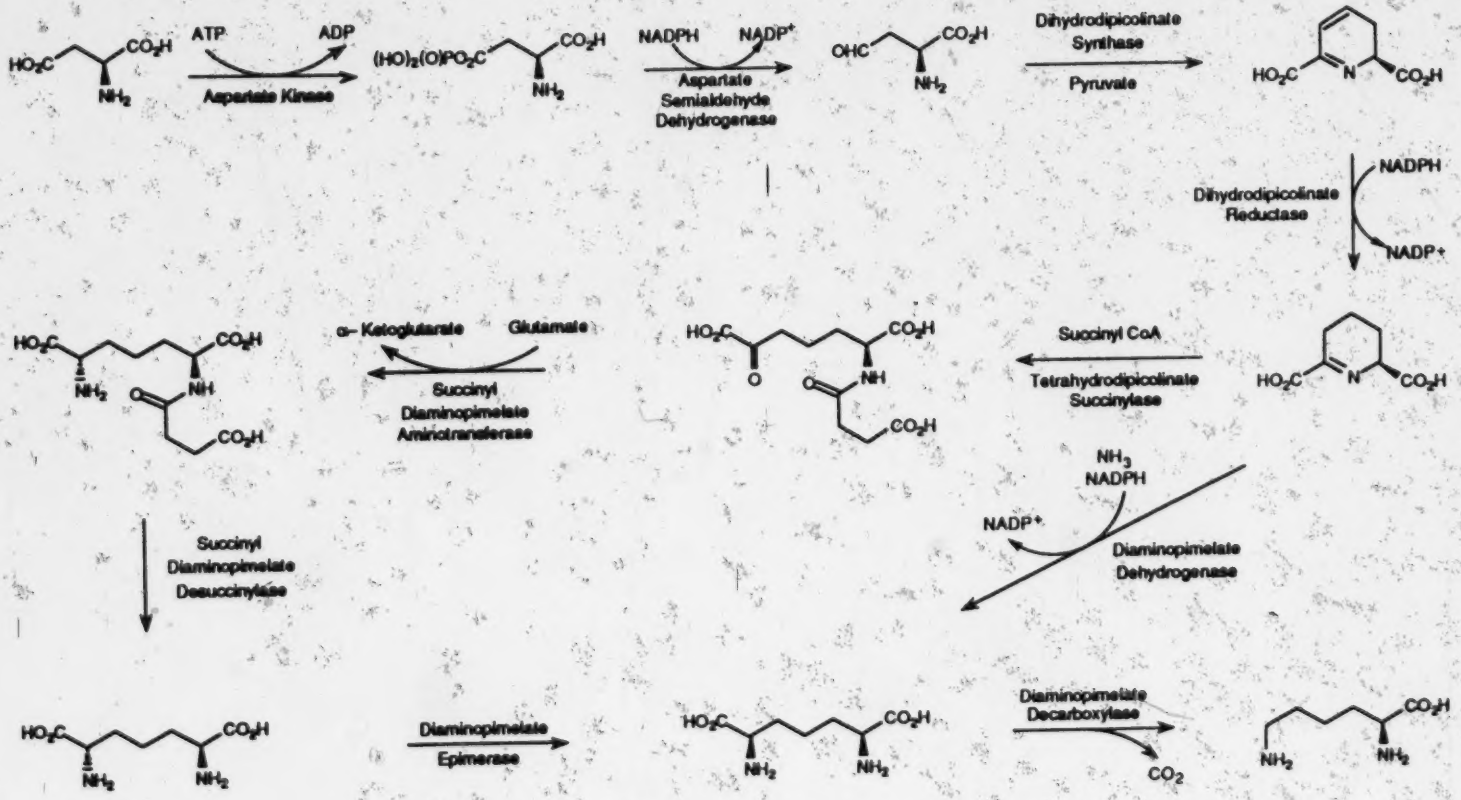


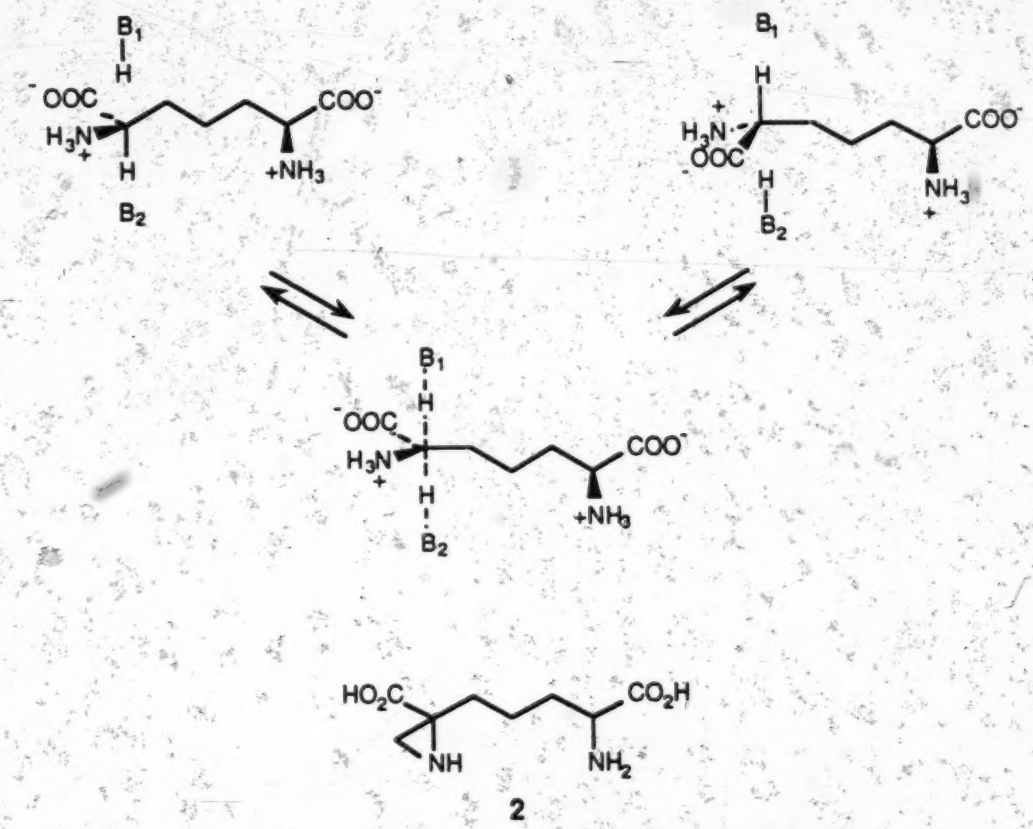
Figure 3. Lysine biosynthesis

Lysine is a required amino acid in mammals,[50] and DAP is not a constituent in animal tissue. Furthermore, DAP and DAP-containing peptides are rapidly excreted.[51, 52] This suggests that inhibitors of lysine biosynthesis could produce antibiotics with minimal side effects in animals and humans. These inhibitors would interfere with lysine production and also would affect cell growth by interfering with DAP and peptidoglycan biosynthesis. One naturally occurring dipeptide antibiotic containing an α-hydroxymethyl analog of DAP is known[53] and in recent years several laboratories have shown that compounds interfering at different stages in lysine and DAP biosynthesis possess antibacterial activity.[36, 38, 41, 54-61]

A prime focus of the Vederas laboratory has been to design and prepare inhibitors for three key enzymes in lysine biosynthesis: diaminopimelate epimerase, diaminopimelate dehydrogenase, and diaminopimelate decarboxylase.

Diaminopimelate epimerase, which was first detected over 30 years ago,[62] has been purified and characterized by Wiseman and Nichols in 1984,[40] and more recently has been overproduced and purified to apparent homogeneity by Higgins and coworkers.[41] This enzyme has a molecular weight of 34,000 and is a non-pyridoxal phosphate (PLP) dependent racemase which requires the presence of at least one L center in its substrate.[62] The enzyme contains an active-site thiol [40] and its mechanism[40, 41] is believed to resemble the two-base mechanism of proline racemase[63] (Figure 4). Confirmation of the presence of an active-site thiol was recently shown by the action of the mechanism-based irreversible inhibitor AZIDAP (2)[41, 59] (Figure 4). Isolation of modified peptides following inactivation indicated cysteine 73 was covalently bound to the inhibitor.[41] The gene for the epimerase has been sequenced[64] and cloned[65] and the N-terminal sequence determined for the first 5 amino acids in the protein are in agreement with the nucleotides in the gene.[64] This enzyme is thought to act as a monomer, although more recently it has been proposed to be a dimer of identical subunits.[41, 59]

Figure 4. Proposed Mechanism for DAP Epimerase and Structure of AZIDAP



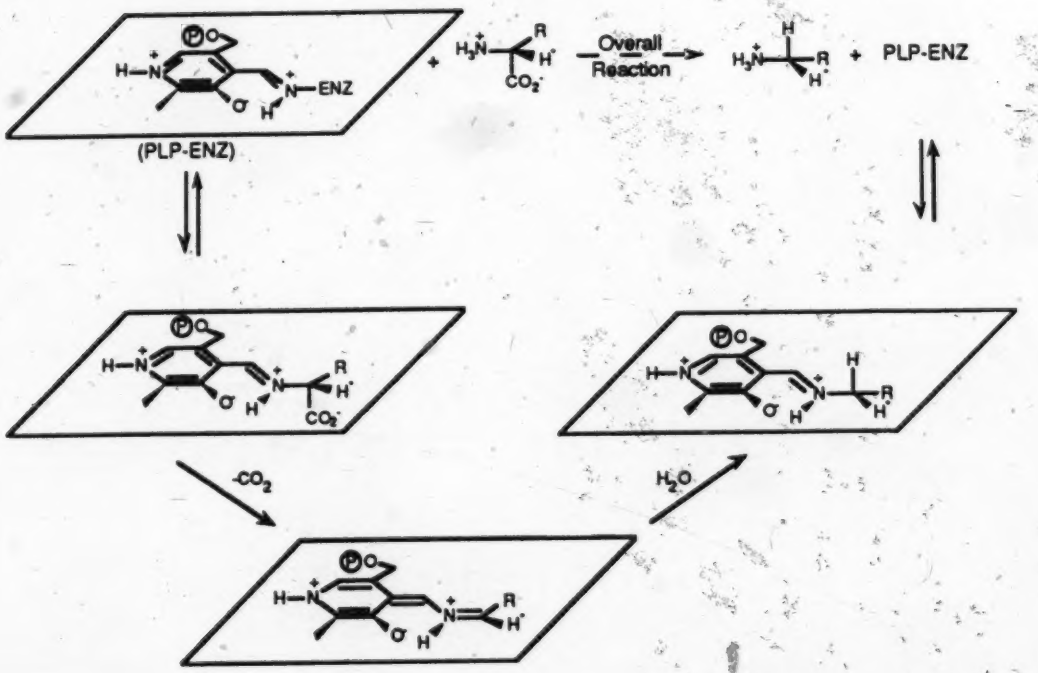
2

Diaminopimelate dehydrogenase is an unusual nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent dehydrogenase in that it operates on the D center of DAP.[49] This 80,000 dalton enzyme is composed of two identical subunits of molecular weight 40,000,[66] and shows a strict substrate specificity for the *meso* isomer of DAP[55, 66] and contains a non-essential active site thiol[67] and an active site tryptophan.[66] The protein has been purified and characterized from several bacteria[44, 46, 66, 68] and the gene has been cloned and sequenced from *Corynebacterium glutamicum*.[69]

Diaminopimelate decarboxylase has been isolated from a number of

plant[22, 24, 70] and bacterial sources.[5, 43, 71-74] This PLP-dependent decarboxylase of molecular weight 200,000, like the dehydrogenase, also acts on the D center of DAP, and decarboxylates with inversion of configuration[75, 76] (Figure 5). Also like the dehydrogenase, this protein has a strict substrate specificity for *meso* -DAP, with neither the L,L nor the D,D isomers functioning as a substrate or inhibitor.[43] The gene for this enzyme has been sequenced from bacterial sources.[77-79]

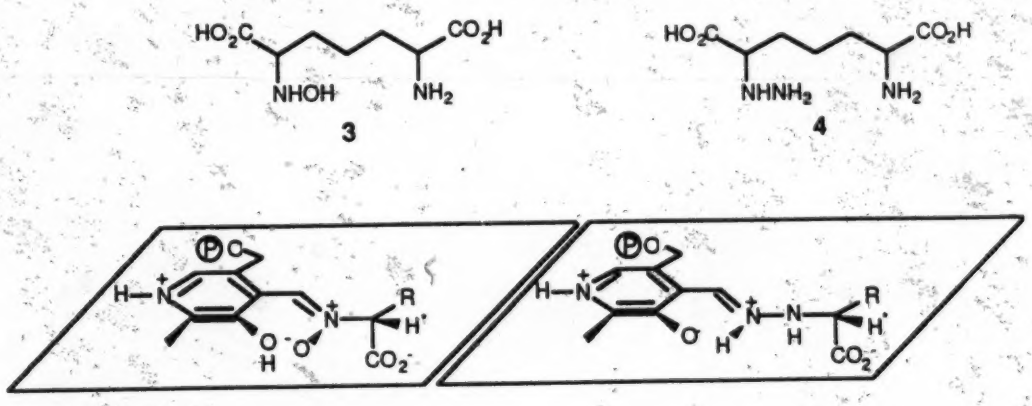
Figure 5. Proposed Mechanism of DAP Decarboxylase



Previous work in our research group by Dr. Janice Kelland[80] had involved the synthesis of *N*-hydroxy-DAP **3** and *N*-amino-DAP **4** (mixtures of all possible isomers) as potential inhibitors for DAP decarboxylase.[57] It was envisioned that these inhibitors would bind to the cofactor and produce a nitrone[81] or hydrazone[57] intermediate, respectively, which would be more stable to hydrolysis than the ordinary aldimine intermediate (Figure

6). When these two compounds were tested against DAP decarboxylase isolated from wheat germ and *Bacillus sphaericus* it was found that these N-modified analogs were moderate competitive inhibitors, 4 being almost an order of magnitude more effective than 3.[57] Antibacterial testing showed that these analogs effectively inhibited certain bacteria.

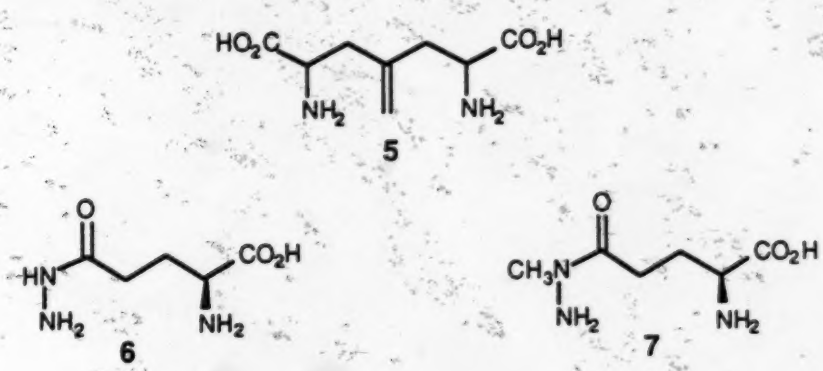
Figure 6. *N*-Hydroxy-DAP and *N*-Amino-DAP and Their Proposed Mode of Inhibition of DAP Decarboxylase



However, work by Girodeau and coworkers[60] on a series of olefinic analogs, designed to inhibit the decarboxylase, indicated that although 4-methylene DAP (5) (Figure 7) was a weak inhibitor of bacterial DAP decarboxylase, it possessed potent antibacterial properties. Separation of the isomers and subsequent testing determined that the individual stereoisomers displayed identical antibacterial activity to the original mixture. These results suggested that DAP decarboxylase may not be the point of action for this analog but perhaps DAP epimerase was the main enzyme inhibited. Both these enzymes have mechanisms that involve a stabilized carbanionic intermediate and therefore could be inhibited by amino acid derivatives containing the same potentially reactive groups.[60] Thus it was decided to prepare the *N*-amino- and *N*-hydroxy-DAP (as mixtures of all possible isomers) and investigate their effect on DAP epimerase. In addition to these nitrogen-modified analogs, two other potential inhibitors, γ-glutamyl hydrazide (6) and

N'-methyl-γ-glutamyl hydrazide (7) were of interest (Figure 7). These compounds could be possible transition state analogs for the epimerase by virtue of the planar amide nitrogen at the position corresponding to the α-carbon of DAP.

Figure 7. 4-Methylene DAP and the γ-Glutamyl Hydrazides



While 3 and 4 would be useful for initial studies on DAP epimerase, the substrate specificity of this enzyme[62, 82] as well as that of the decarboxylase[43] and dehydrogenase[55, 66] dictates that potentially only one or two of the stereoisomers present in the mixture may be the active inhibitor. Therefore it would be beneficial to have chiral syntheses of these analogs. In the previous preparations of these nitrogen-modified compounds, 4 was prepared by a chiral amination step, while 3 was made by oxidation of a selectively protected DAP moiety.[57] DAP itself has not yet been synthesized asymmetrically, but its various isomers have been obtained either by fermentation and isolation,[83, 84] or by the tedious multistep separation from a mixture of all possible isomers.[85-87] Hence we chose DAP itself as the initial target for asymmetric synthesis, hoping to design a route by which other analogs and selectively protected DAP moieties could be readily available as well. Selectively protected DAP compounds would be useful in the synthesis of various DAP-containing peptides, a number of which have shown potent immunomodulatory[84, 88-111] and sleep-promoting effects.[52, 112-117]

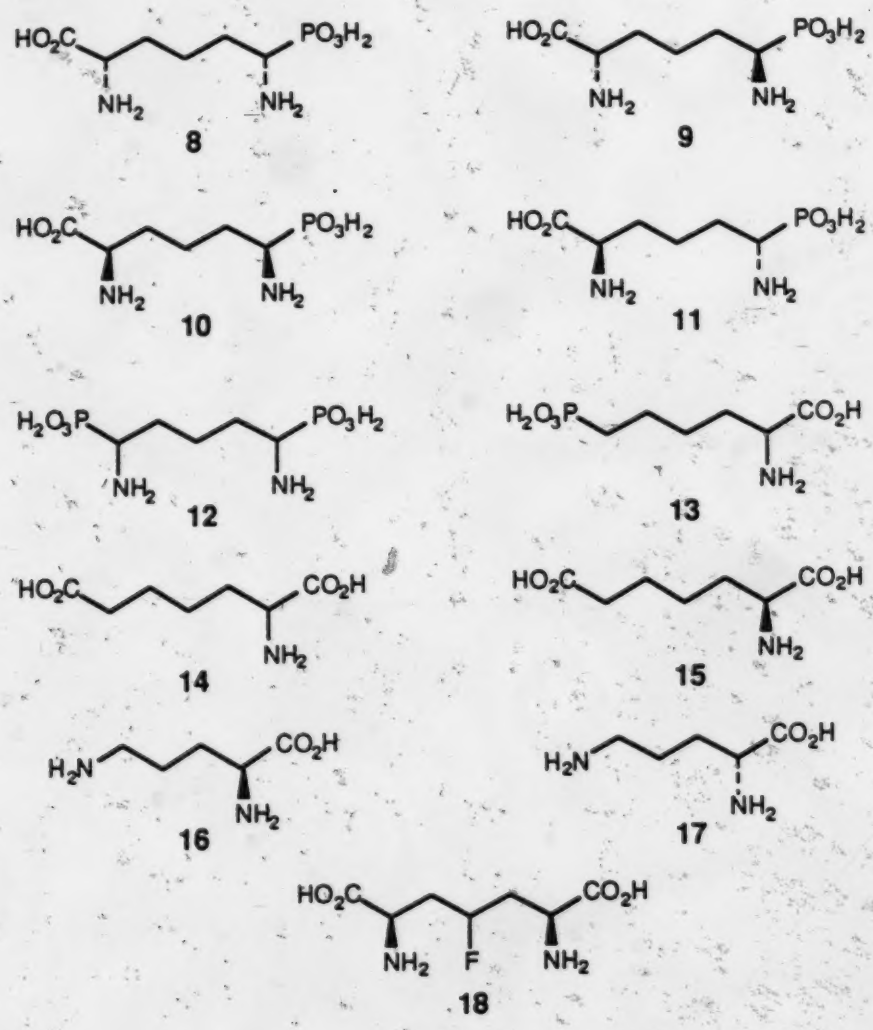
The asymmetric synthesis of amino acids is the subject of a great number of papers in the recent literature.[118, 119] The majority of these methods utilize one of several general approaches: stereoselective alkylation,[120-130] asymmetric hydrogenation,[131-136] stereoselective carboxylation,[137] stereoselective amination,[138-141] Michael addition,[142] enolate-aldehyde condensation for the preparation of β-hydroxy α-amino acids,[143-145] and various rearrangements.[146-150] Finally, stereoselective halogenation, hydroxylation or epoxidation with subsequent transformation to the amine have also been employed.[141, 151-157]

Another class of amino acid analogs, the α-hydrazino acids, display a wide range of biological activities.[158-181] These compounds can also be cleaved to α-amino acids,[182-184] thereby providing another route to these materials. However, fewer methods exist for their preparation. Many of the earlier routes[162, 168, 183-195] suffer from low yields, multi-step procedures, or loss of optical purity from the starting amino acid. One successful approach for the stereoselective synthesis of these compounds is the amination of chiral enolates by azodicarboxylates.[196-201] Another useful procedure is displacement of a triflate with an appropriately protected hydrazine.[181]

From the literature examined above, the most general approach for the chiral synthesis of DAP and its analogs appeared to be formation of an α-hydroxy compound with further elaboration to the α-amino- or α-hydrazino acid. Development of this method will be described later.

Another potential class of inhibitors for the DAP enzymes are compounds in which one or both of the carboxyl groups of DAP are replaced by a phosphonate (Figure 8).

Figure 8. Substrate Analogs with Potential for Inhibition of DAP Epimerase



Phosphorous modified amino acids as well as peptides have been used successfully and extensively as enzyme inhibitors, particularly as transition-state analogs.[202-214] The phosphonate analog of alanine is a potent inhibitor of the PLP-dependent enzyme alanine racemase,[215-219] as is the β-chlorophosphonate analog of alanine.[209] Thus the phosphonate analogs of DAP **8-12** could be potential inhibitors of the PLP-dependent DAP decarboxylase and DAP epimerase. In the case of the epimerase, the doubly charged

phosphonate dianion may allow for tighter binding in the active site, whereas the additional negative charge may also influence the function of the two bases in the active site. A review of the literature on DAP epimerase also revealed that, with the exception of lysine (**16**, **17**), a number of other simple amino acids resembling DAP, such as α-aminopimelate (**14**, **15**), 7-phosphono-2-aminoheptanoic acid (**13**), and 4-fluoro-DAP **18**, have not been examined previously as substrates or inhibitors (Figure 8). Thus the inhibition of DAP epimerase by these amino acids and the phosphonate analogs was investigated. The monophosphonate analogs **8-11** and the 4-fluoro-DAP **18** were prepared in stereochemically pure form, and the diphosphonate compound **12** as a mixture of isomers, by Dr. Yonghong Song in our research group. The results of this study should provide a greater insight into the specificity and active-site requirements of the epimerase.

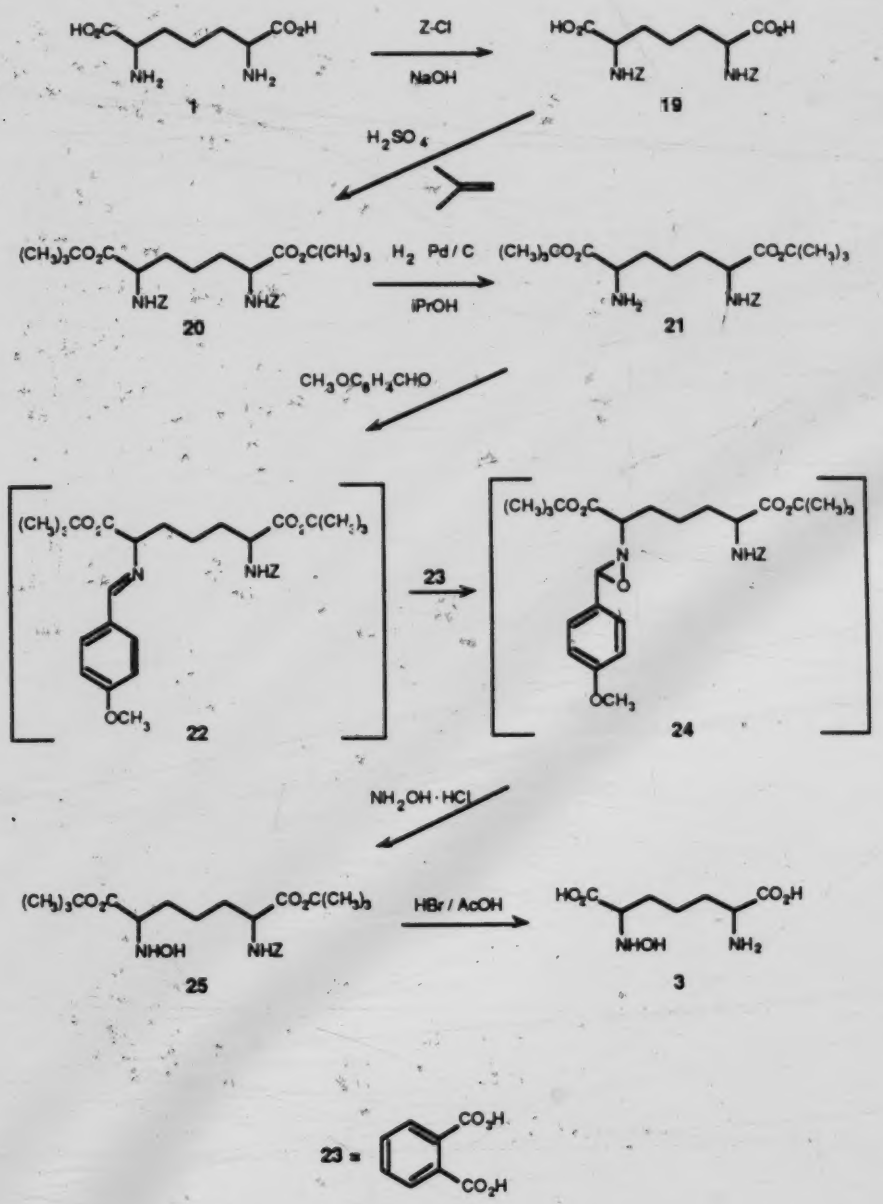
Results and Discussion

The proposal by Girodeau and coworkers[60] that the antibiotic activity of some DAP analogs may be due to inhibition of DAP epimerase rather than DAP decarboxylase prompted the further investigation of the analogs *N*-hydroxy DAP (**3**) and *N*-amino DAP (**4**) as inhibitors of the epimerase. Preparation of these compounds was achieved using the routes previously developed by Dr. Janice Kelland.[57, 80] For the *N*-hydroxy compound **3**, the route shown in Scheme 1 was followed.

Commercially available DAP (statistical mixture of all possible stereoisomers) was treated with benzyl chloroformate (Z-Cl) in aqueous sodium hydroxide to afford the dicarbobenzyloxy (di-Z) derivative **19** in 99% yield. Esterification by a modification of the method of Bricas and coworkers[220] gave an 82% yield of the di-*t*-butyl ester **20**. Alternative methods for the diesterification, such as transesterification with *t*-butyl acetate in the presence of perchloric acid,[221] or formation of the acid chloride with oxalyl chloride and subsequent treatment with *t*-butyl alcohol, were unsuccessful. The mono-Z-di-*t*--butyl ester **21** was obtained by hydrogenation in isopropyl alcohol over 5% palladium on carbon in 40% yield. The deprotection was also attempted using trimethylsilyl iodide[222] with no improvement over the results obtained by hydrogenation.

The procedure of Polonski and Chimiak[223] allowed the oxidation of the amino group to the hydroxylamino moiety. The imine **22**, formed *in situ* from *p*-methoxybenzaldehyde, was oxidized by monoperphthalic acid (**23**) to give oxaziridine **24**, which was not isolated. Subsequent opening of the oxaziridine by hydroxylamine hydrochloride produced the protected *N*-hydroxy amino acid **25** in 29% yield. This compound was then deprotected using hydrogen bromide in acetic acid to give a 98% yield of the desired *N*-hydroxy amino acid **3**.

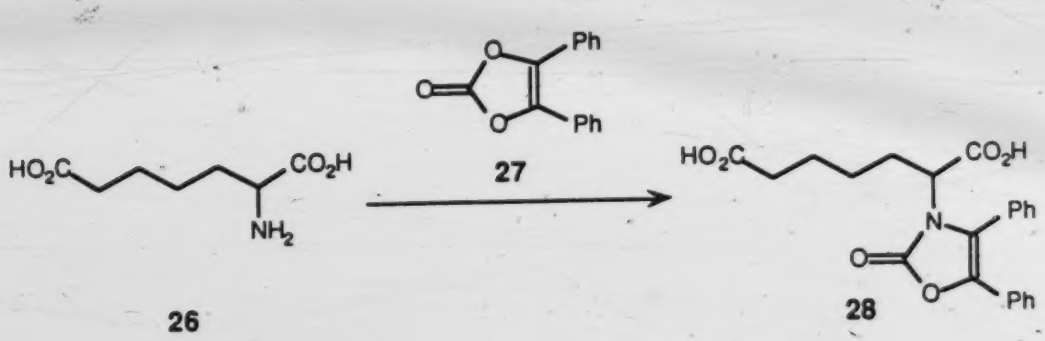
Scheme 1



For the preparation of the hydrazino amino acid analog **4**, commercially available D,L α-aminopimelic acid (**26**) was protected by the method of Sheehan and Guziec [224] (Scheme 2). The 4,5-diphenyl-1,3-dioxol-2-one (**27**) was prepared in 34% yield from

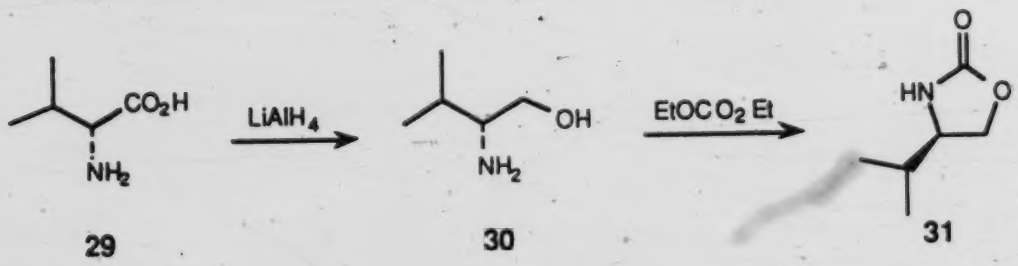
benzoin. Reaction of this dioxolone with α-aminopimelic acid gave the protected amino acid **28** in 70% yield.

Scheme 2



To direct the subsequent amination and protect the free acid groups of **28**, the condensation of the oxazolidinone[225] **31** with diacid **28** was required. This chiral auxiliary was prepared by treatment of valinol[226] (**30**) (obtained by reduction from D-valine (**29**) in 64% yield) with diethyl carbonate to give the oxazolidinone **31** (87% yield) (Scheme 3).

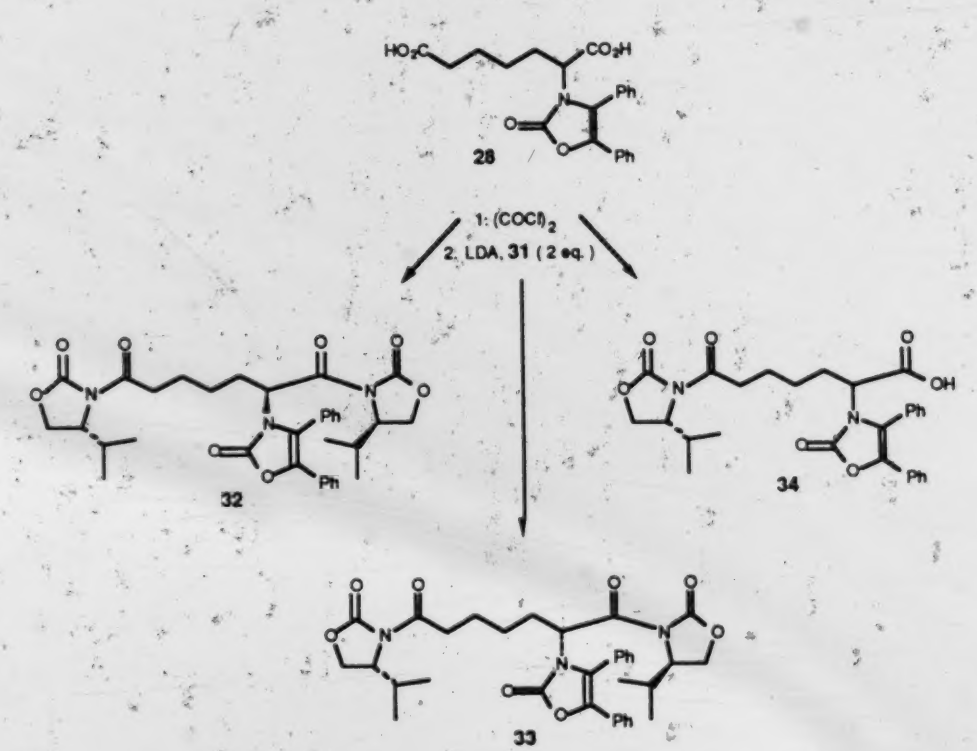
Scheme 3



Reaction of **28** with oxalyl chloride,[227] followed by treatment of the crude acid chloride obtained with two equivalents of the lithium salt of **31** gave compounds **32**, **33**,

and **34** in 28, 31, and 33% yields, respectively, after separation by flash chromatography (Scheme 4).

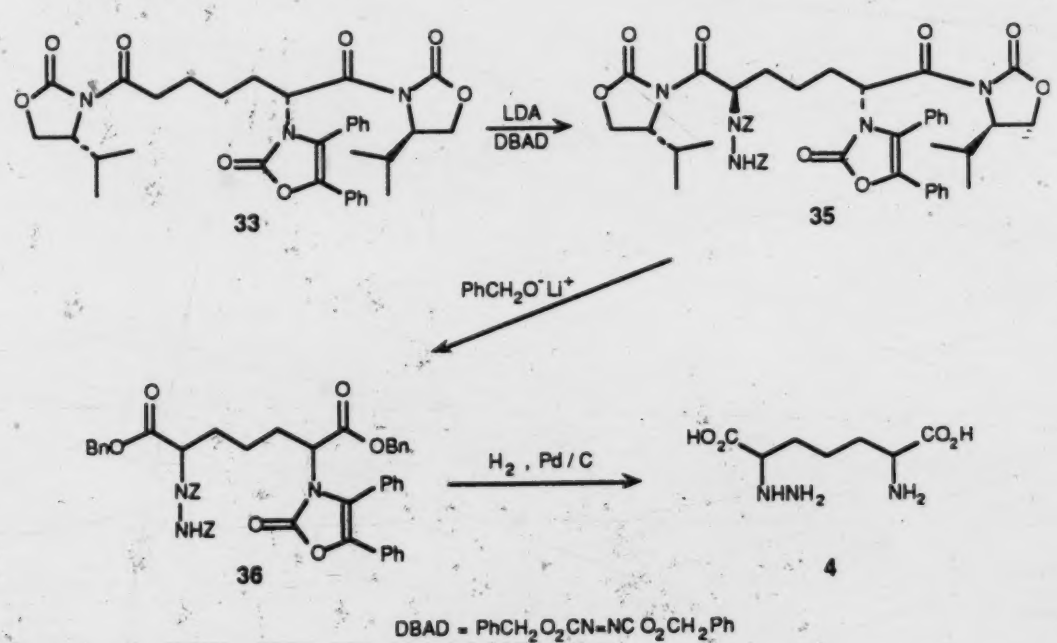
Scheme 4



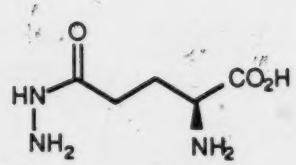
Previous work[80] indicated that amination to give the desired hydrazino compound would only proceed on isomer **33** under the conditions used here. Therefore this compound was treated with lithium diisopropylamide (LDA) followed by dibenzyl azodicarboxylate, to yield 29% of the aminated product **35**. Since it had been shown that racemization of this material occurs upon removal of the chiral auxiliaries with lithium benzyloxide treatment at 0 °C,[57, 80] several other hydrolysis procedures were investigated. Treatment of **35** at -78 °C with lithium benzyloxide for several days as described by Evans and coworkers,[197] failed to reduce the amount of racemization. Similarly, hydrolysis with

lithium hydroperoxide[200] or reaction with lithium hydrosulfide and subsequent treatment with peracetic acid[228] were unsuccessful. At this point further exploration of the hydrolysis was abandoned and the mixture of all possible isomers was generated by the reaction of lithium benzyloxide at 0 °C to give dibenzyl ester **36** in 52% yield. Hydrogenation and ion exchange chromatography afforded *N*-amino-DAP (**4**) [57] in quantitative yield (Scheme 5).

Scheme 5



The next targets chosen were γ-glutamyl hydrazide (**6**) and *N'*-methyl-γ-glutamyl hydrazide (**7**). These materials could function as transition-state analogs since they have



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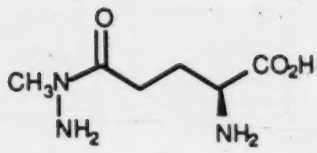

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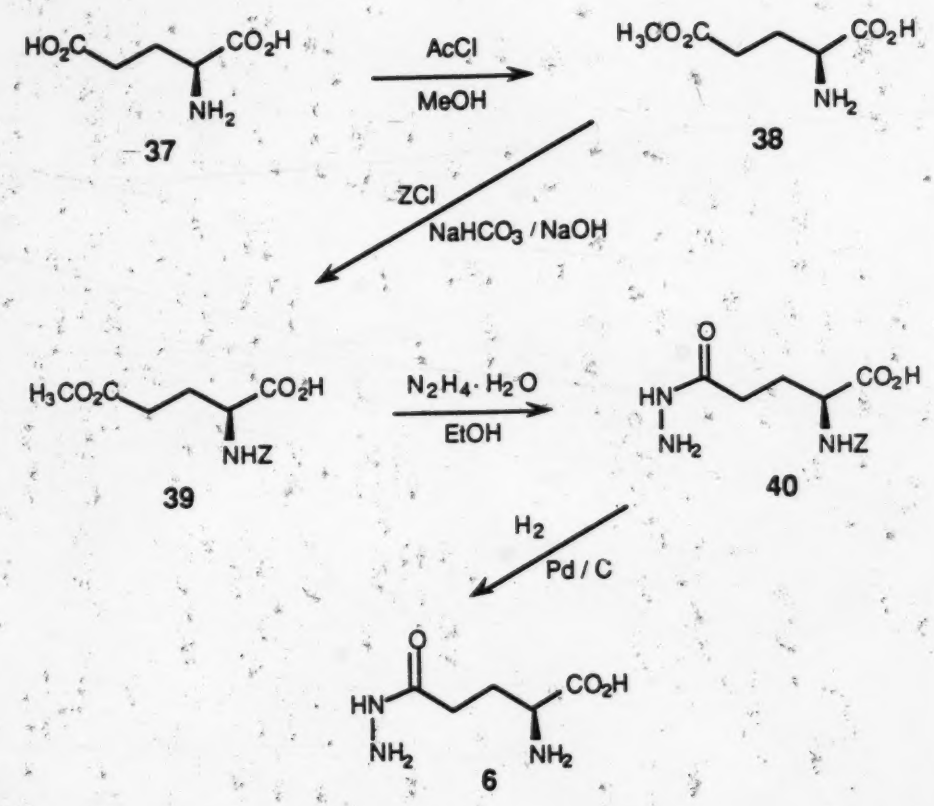
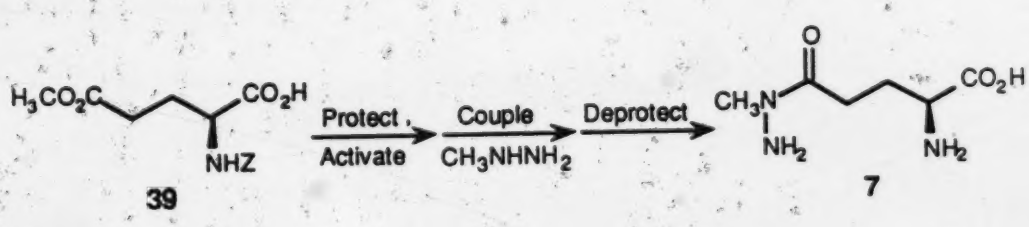
a planar amide nitrogen at the position which would correspond to the α-carbon of DAP. It is this carbon in DAP which is postulated to go through a planar intermediate during epimerization. γ-Glutamyl hydrazide is commercially available and has a wide variety of biological effects. This compound is a known antibacterial agent[229, 230] due to its interference with glutamine metabolism,[230-233] and its inhibition of some transport systems.[234-236] However, it has also been shown to interfere with mammalian glutamate metabolism.[237-245] The hydrazide has also found use in peptide and amino acid synthesis.[246] *N'*-methyl-γ-glutamyl hydrazide (**7**) has been examined as a potential product in the reaction of glutamate and methylhydrazine catalyzed by glutamine synthetase,[247] but no examination of its biological effects has been done. The synthesis of these two compounds was readily achieved using literature procedures.

L-Glutamate (**37**) was esterified at the γ-position through the action of acetyl chloride and methanol[248] to give a 41% yield of the γ-methyl ester **38**. Protection of the amine by treatment with benzyl chloroformate in aqueous sodium bicarbonate and sodium hydroxide gave the nitrogen-protected mono-acid **39** in 77% yield. This acid ester reacts with hydrazine hydrate to give the α-amino protected hydrazide **40** in 81% yield. Hydrogenation affords a 77% yield of the γ-glutamyl hydrazide (**6**) (Scheme 6).

Scheme 6



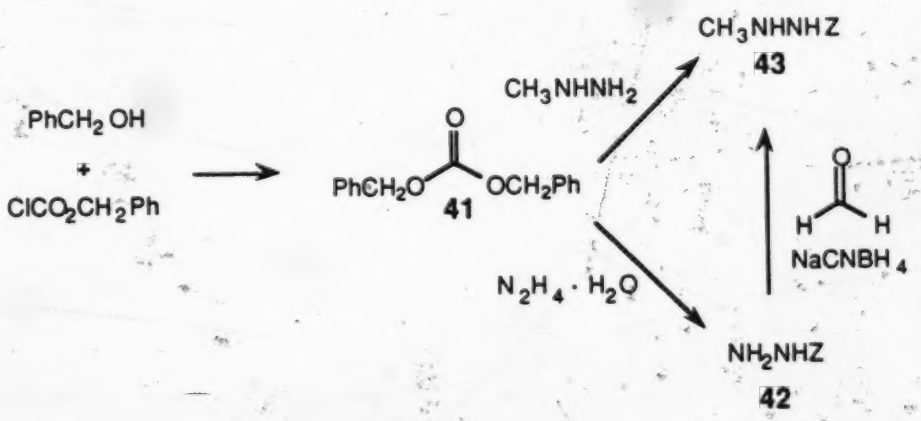
To prepare the substituted glutamyl hydrazide 7, the half ester derivative 39 required further protection and activation before coupling with methylhydrazine.



Furthermore, masking of the primary amine of the hydrazine was necessary to ensure reaction at the methylated nitrogen. The required hydrazine moiety could be made either by protection of hydrazine followed by methylation, or by protection of methyl hydrazine

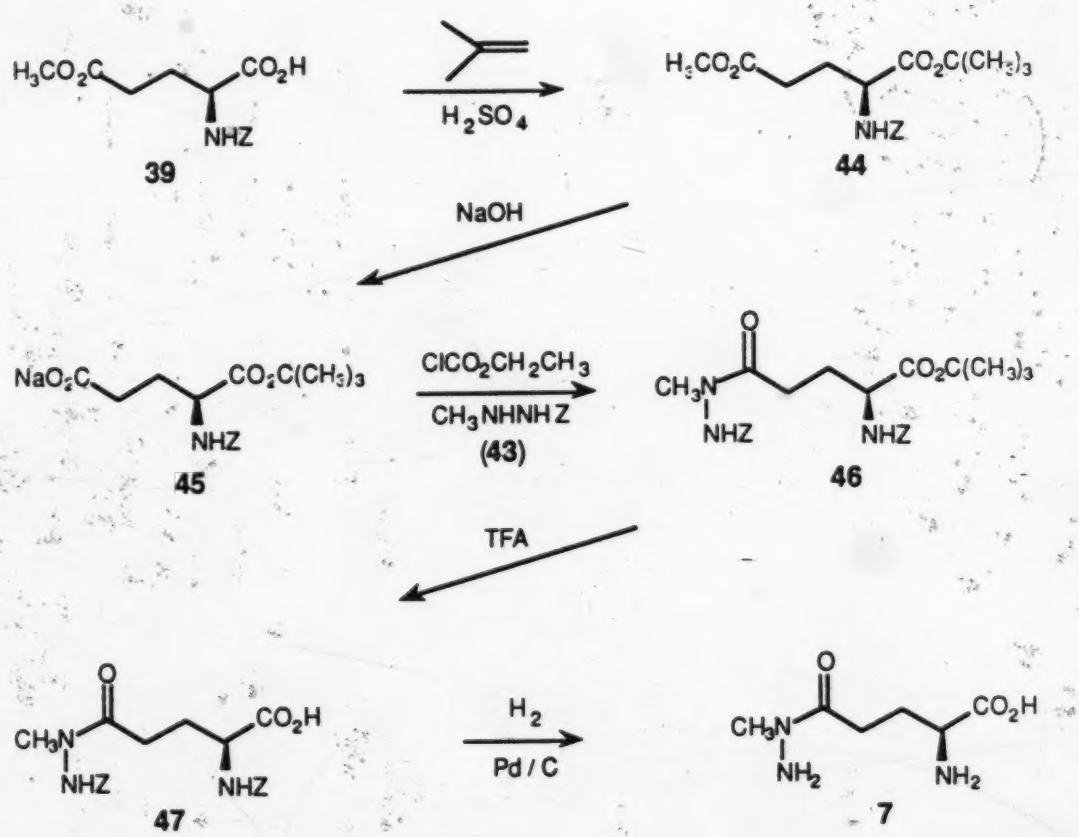
directly. It was found that protection of hydrazine by reaction with dibenzyl carbonate (**41**), prepared in 37% yield from benzyl chloroformate and benzyl alcohol, resulted in a 14% yield of the Z-hydrazine **42**. Methylation with formaldehyde in the presence of sodium cyanoborohydride,[249] gave a 61% yield of the desired *N*-methyl- *N'*-Z hydrazine **43** (overall yield of 12%). Reaction of methylhydrazine with dibenzyl carbonate results in an easily separable mixture of regioisomers, generating **43** directly in 19% yield (Scheme 7).

Scheme 7



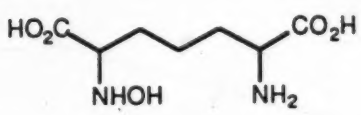
The α-carboxyl of **39** was esterified with isobutylene and sulfuric acid[220] to give the mixed diester **44** in 88% yield. Diester **44** was hydrolyzed with one equivalent of sodium hydroxide to remove the methyl ester and give the sodium salt **45** in 81% yield. Activation of the free carboxyl group by treatment with ethyl chloroformate and reaction with **43** afforded a 51% yield of the protected methyl glutamyl hydrazide **46**. Removal of the *t*-butyl ester with trifluoroacetic acid (TFA) to give intermediate **47** in 88% yield, followed by hydrogenation,[247] generated the desired methyl glutamyl hydrazide **7** in 70% yield (Scheme 8).

Scheme 8



With the syntheses of the target compounds **3**,**4**,**6**, and **7** successfully completed, enzyme inhibition studies with these compounds were initiated. These experiments were done in collaboration with Dr. Monica Palcic and Dr. Lister Lam.[55] It was necessary to test the DAP dehydrogenase first, as this enzyme is commonly used in a coupled assay for DAP epimerase.[40, 55] Of these compounds, the two hydrazides **6** and **7** did not detectably act as substrates for the dehydrogenase. However, the nitrogen-modified analogs **3** and **4** did behave as substrates for this enzyme, having relative velocities of 22% and 4% of the natural substrate, *meso*-DAP, respectively. None of these analogs showed time-dependent

inhibition against the dehydrogenase and DAP epimerase, thereby indicating none of these compounds was an irreversible inhibitor.



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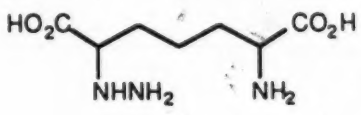

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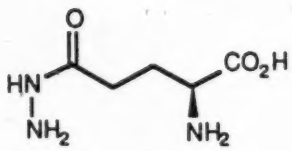

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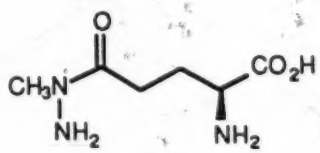

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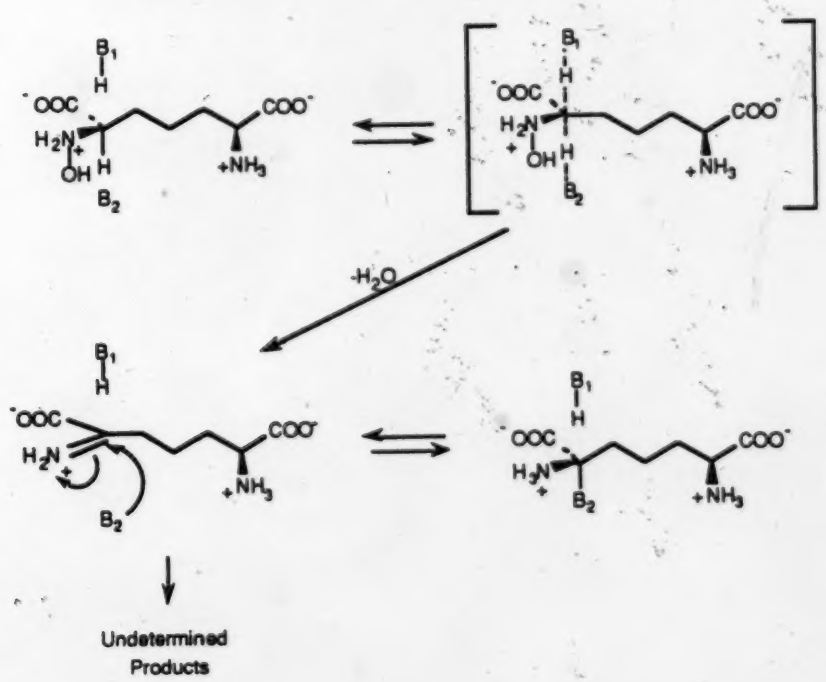
N-Hydroxy DAP was found to be an extremely potent competitive inhibitor for the epimerase. To confirm that the inhibition was due to analog **3** only, and not the product formed by its turnover by the dehydrogenase present in the coupled assay, **3** was tested against the epimerase alone using a radioactive assay.[40, 55] Results indicated that the inhibition of the epimerase was due to the *N*-hydroxy analog and not any turnover product. Furthermore, preincubation of the analog with the dehydrogenase under various conditions did not affect the inhibitory effect of the compound on DAP epimerase, thereby suggesting that the isomer which acts as a substrate for the dehydrogenase is not the same isomer which inhibits the epimerase. In contrast, *N*-amino DAP (**4**) was found to be only a modest competitive inhibitor of the epimerase. The glutamyl hydrazide analogs **6** and **7** did not significantly inhibit the epimerase (Table 1).

Table 1. Inhibition of DAP Epimerase by Analogs 3,4,6 and 7

Analog	Concentration tested (*mM*)	$K_m^{(I)}$ (*mM*)	K_i (*mM*)
3	0.008, 0.4	0.56 ± 0.04	0.0056
4	0.4, 3.2	0.37 ± 0.04	2.9
6	4.0	0.26 ± 0.01	No inhibition
7	4.0	0.28 ± 0.01	No inhibition

The potent competitive inhibition of the epimerase by the *N*-hydroxy analog may be due to elimination of water from the inhibitor in the active site of the enzyme. This would form a planar imine which could act as a potential transition state analog and be attacked reversibly by an active site thiol before its eventual release from the protein [55] (Figure 9).

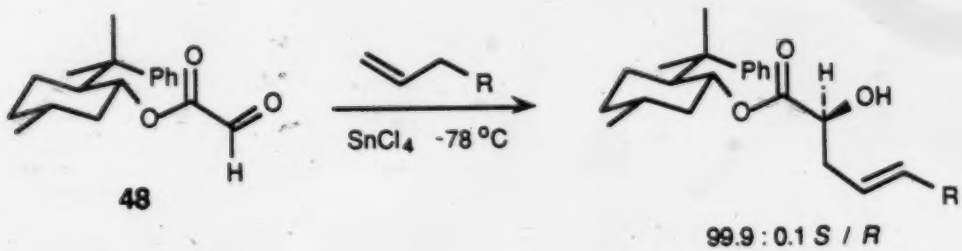
Figure 9. Possible Mechanism of Inhibition for *N*-Hydroxy Analog 3



The potent inhibition of the epimerase by the *N*-hydroxy analog **3** as a mixture of isomers prompted the development of a chiral synthesis of this compound and similar analogs. Since derivative **3** was prepared by the oxidation of a selectively protected DAP moiety, pure stereoisomers of DAP and its selectively protected derivatives appeared to be attractive initial targets.

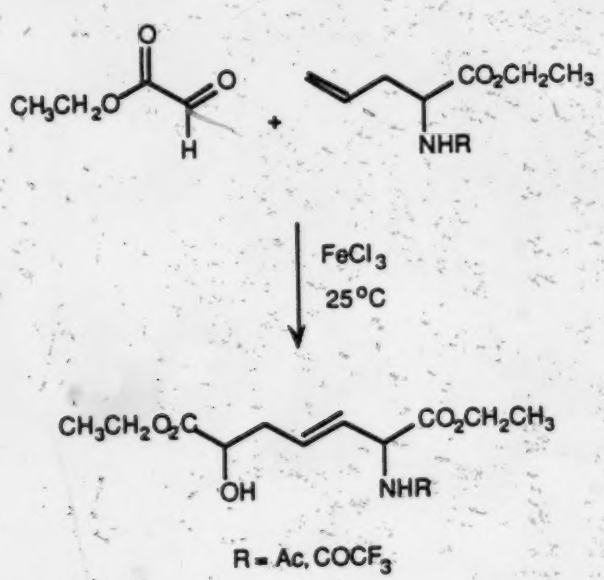
Recent publications by Whitesell and coworkers[250-253] demonstrate that the Lewis acid-catalyzed ene reaction between chiral (-)-8-phenylmenthyl glyoxylate (**48**) and various alkenes provides α-hydroxy esters with high levels of stereochemical induction with the *S* isomer being formed preferentially. This is due to the phenyl group blocking the back face of the glyoxylate, which is held in the *cis* conformation by complexation to the Lewis acid[250, 251, 254-256] (Figure 10).

Figure 10. Asymmetric Induction in Ene Reaction with 8-Phenylmenthyl Glyoxylate



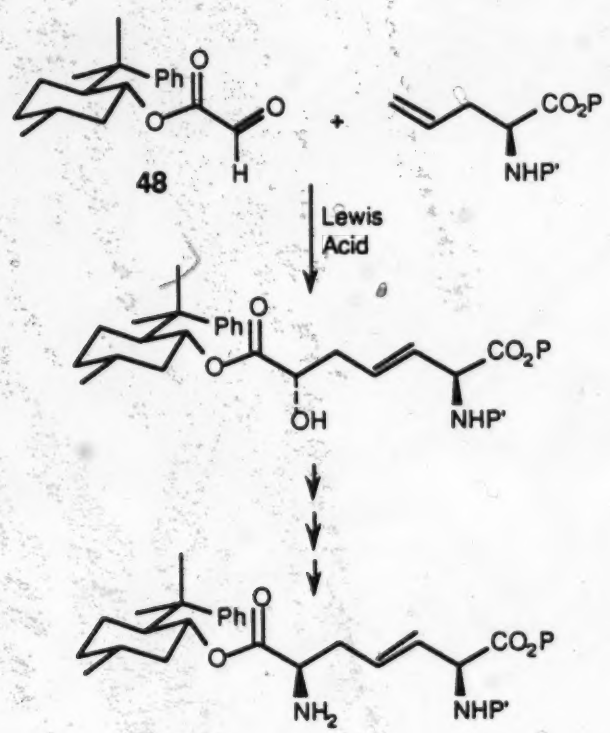
Use of a suitably functionalized alkene, such as a protected allylglycine, in this ene reaction would construct the necessary seven carbon framework of DAP while allowing control of the stereochemistry in the allylglycine moiety. The stereochemistry at the newly formed hydroxyl group would be determined by the enantiomer of 8-phenylmenthol (**53**) used in the preparation of the glyoxylate. Previous work[60, 257] shows that a Lewis acid-catalyzed ene reaction can be used to construct various unsaturated analogs of DAP in racemic form, but an asymmetric synthesis has not been attempted (Figure 11).

Figure 11. Example of Ene Reaction for Assembly of DAP Skeleton



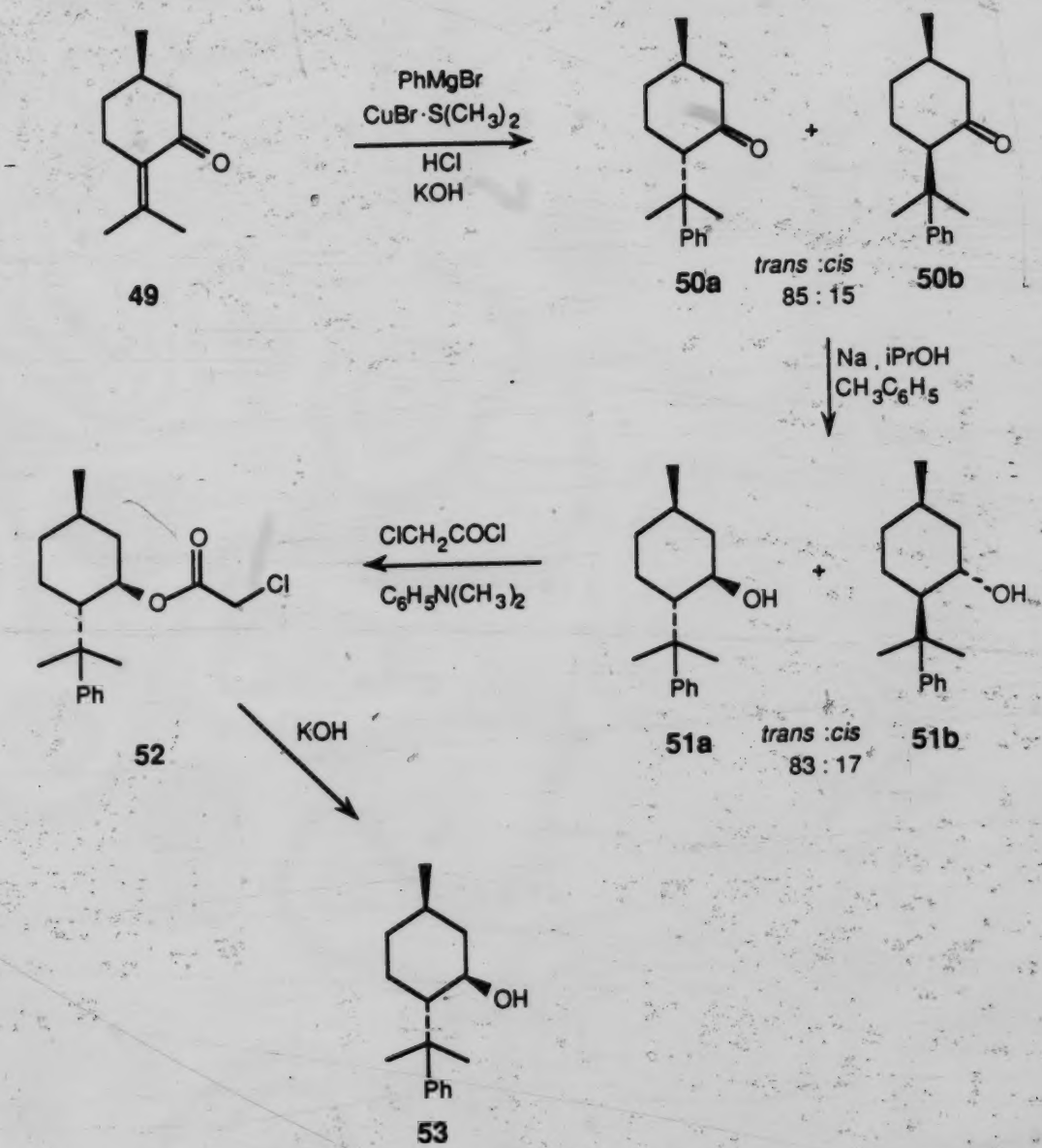
The hydroxyl group produced in the ene reaction can be readily converted to an amine by a number of routes. These include conversion of the hydroxyl moiety to a leaving group such as mesylate or triflate and subsequent displacement with a suitable nucleophile,[60, 181, 257-259] and the Mitsunobu reaction[260, 261] (Figure 12). This approach would also create a selectively protected DAP moiety and, by variation of the nucleophiles used in displacement, could afford access to other analogs modified at nitrogen.

Figure 12. Proposed Synthesis for Protected DAP Intermediate



The (-)-8-phenylmenthol (**53**) and its enantiomer have found use as chiral auxiliaries for asymmetric induction in a wide variety of syntheses. These include Diels-Alder reactions,[262-270] both intramolecular and intermolecular ene reactions,[252, 271-274] cycloadditions,[255, 275-284] hydrogenation,[285, 286] cuprate[287, 288] and other additions,[274, 289-297] cyclizations,[298] reductions,[274, 295] cyclopropanations,[299-303] alkylations,[123, 304, 305] and a number of other reactions.[306-315] Initially (-)-8-phenylmenthol was not commercially available and had to be synthesized by literature procedures[263, 316-319] from (R)-(+)-pulegone. The opposite enantiomer is obtained from (S)-(-)-pulegone, prepared in several steps from citronellol.[262, 263, 320, 321] To prepare this versatile reagent, the method of Ort was followed as outlined in Scheme 9.[319]

Scheme 9



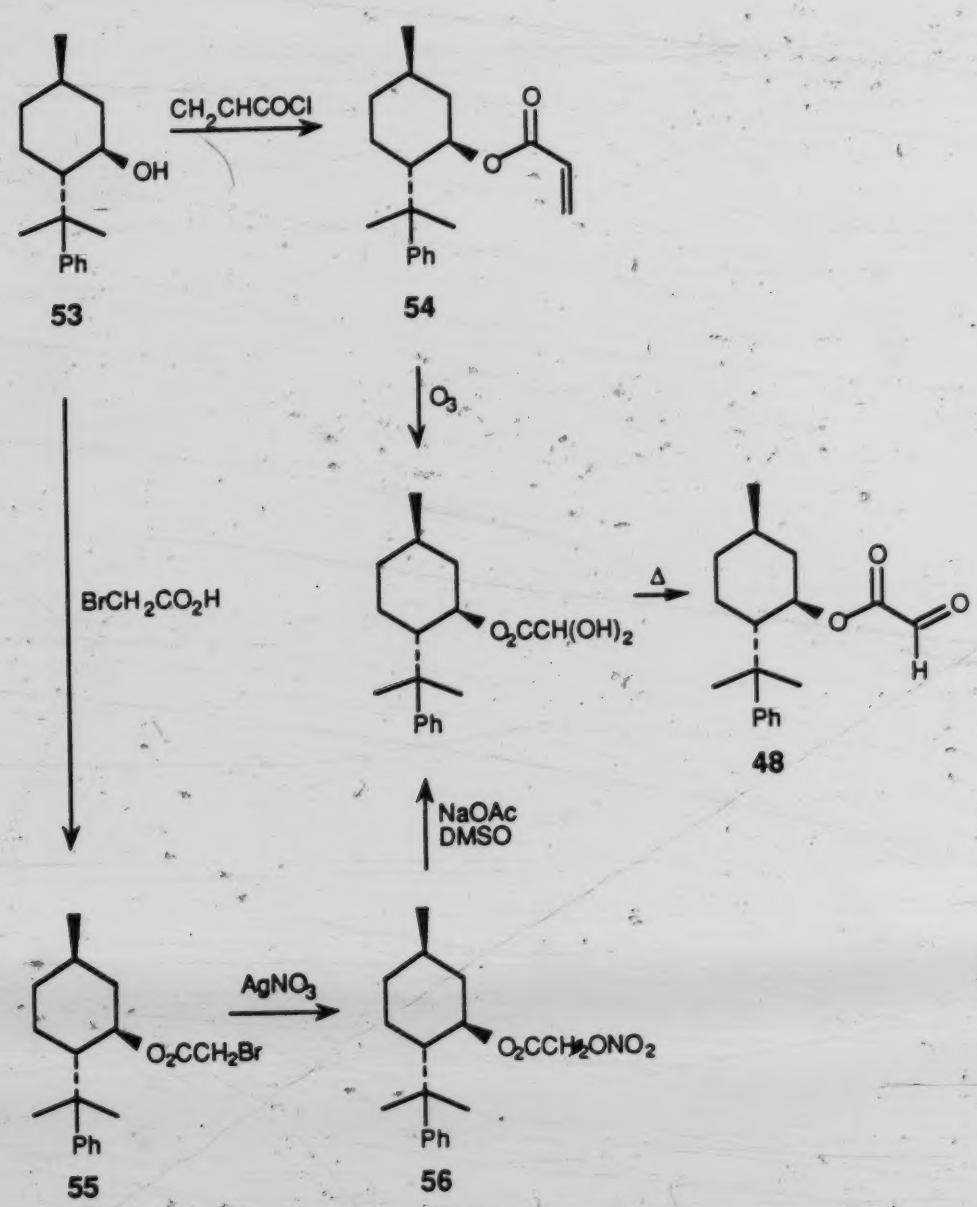
Commercially available (R)-(+)-pulegone (**49**) was treated with freshly prepared phenylmagnesium bromide in the presence of copper bromide dimethyl sulfide complex. Work-up followed by equilibration with refluxing base gave the ketone adducts **50a** and

50b in a yield of 82%. The ratio of the epimers was determined to be 85 :15 *trans* to *cis* on the basis of the integration of the pairs of peaks found for the carbonyl, quaternary carbons, and the methine adjacent to the carbonyl in the inverse-gated ^{13}C NMR spectrum. The mixture of ketones was reduced in the presence of sodium and isopropyl alcohol to the corresponding alcohols **51a** and **51b** in 85% yield. The ratio of these isomers was also determined by integration of pairs of carbon peaks found for the quaternary carbons and the gem-methyl groups in the inverse-gated ^{13}C NMR spectrum, and was found to be 83:17 *trans* to *cis*. Separation of this mixture was achieved by conversion to the chloroacetate and successive recrystallizations, to afford a 66% recovery of chloroacetate **52** . At this stage the optical rotation of this derivative was slightly lower than that reported in the literature.[319] Calculations indicated an enantiomeric ratio of 97 : 3. Hydrolysis of the chloroacetate **52** in base gave a 83% yield of the (-)-8-phenylmenthol (**53**); again the optical rotation was depressed, indicating an enantiomeric ratio of 98 : 2, which is the same as that found for the chloroacetate within experimental error. Examination of the starting (R)-(+)-pulegone (**49**) (96%, $[\alpha]_D$= +22.8 °, calculated $[\alpha]_D$= +23.3 °) indicated that this material was enantiomerically impure (pure pulegone $[\alpha]_D$= +24.6 °[319]). Hence the prepared 8-phenylmenthol (**53**) is also not enantiomerically pure.

Although the synthesized chiral auxiliary was not enantiomerically pure, work on this route was continued. Hence the prepared (-)-8-phenylmenthol (**53**) was converted to the glyoxylate **48**. Two possible routes are available to prepare the glyoxylate, both of which were used successfully. The 8-phenylmenthol (**53**) was treated with acrolyl chloride to create the acrylate ester **54** in 67% yield. Ozonolysis in methanol and dichloromethane with reductive work-up resulted in a 76% yield of the glyoxylate **48**. Alternatively the alcohol **53** was converted to the bromoacetate **55** in 90% yield by esterification with bromoacetic acid in the presence of *p*-toluenesulfonic acid. Treatment with silver nitrate produced the nitrate ester **56** in 87% yield. Subsequent oxidation with

dimethylsulfoxide (DMSO) and sodium acetate via a Swern-type mechanism [322] produced the glyoxylate **48** in 97% yield after dehydration and distillation (Scheme 10).

Scheme 10

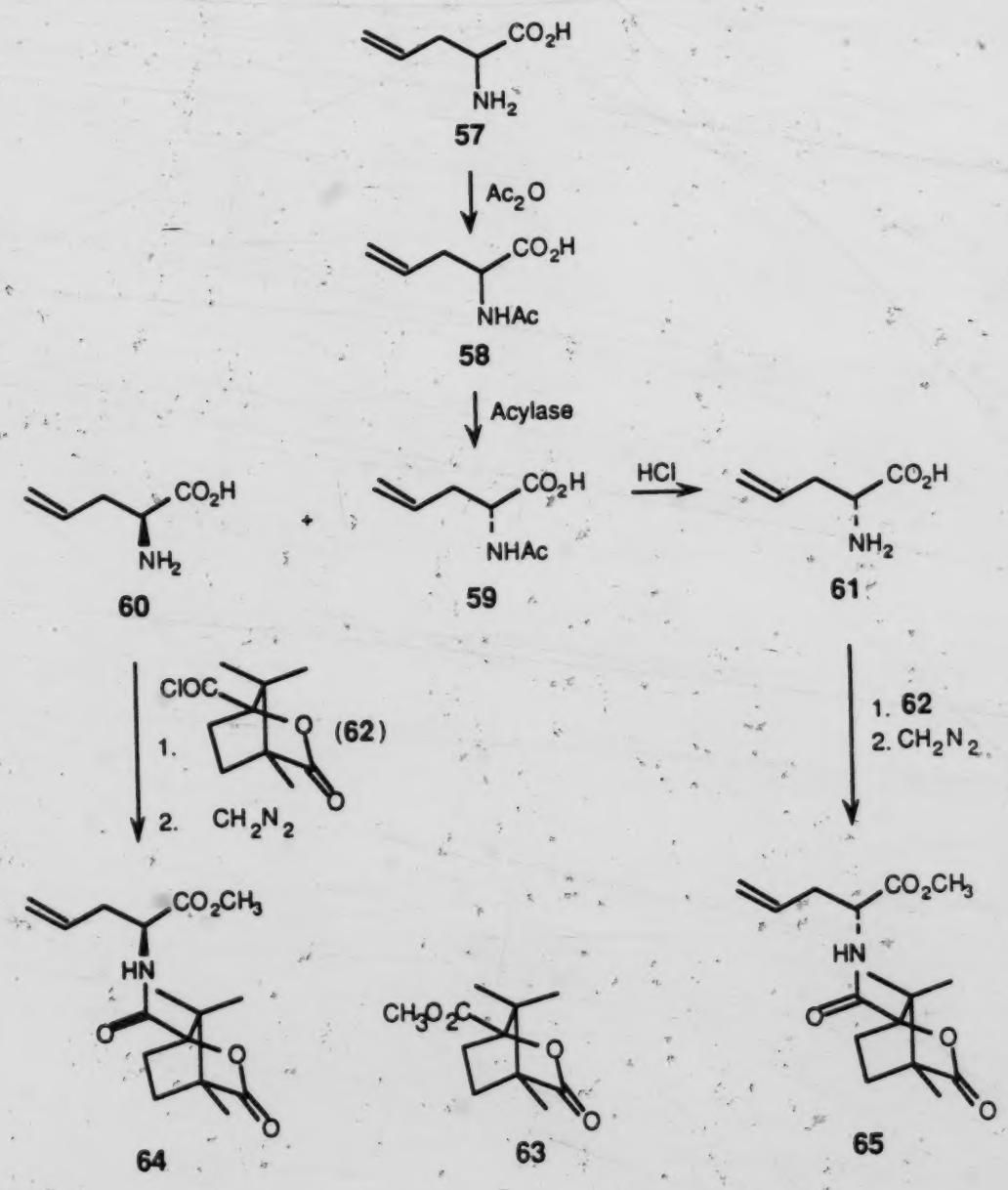


Having successfully prepared the 8-phenylmenthol glyoxylate portion, the required allylglycine derivatives were then obtained by elaboration of the commercially available isomers. Alternatively, the pure isomers may be obtained by resolution of the commercially available racemate. Choosing the less expensive latter method, the D- and L-isomers were resolved from racemic allylglycine (**57**) by the method of Black and Wright.[323] Compound **57** was converted to its acetamide **58** by treatment with acetic anhydride in aqueous sodium hydroxide (96% yield). Incubation of this material with hog kidney acylase I, followed by recrystallization, produced the D-acetamide **59** and the free L-amino acid **60** in 63% and 55% yields, respectively. Hydrolysis of the D-acetamide in aqueous hydrochloric acid afforded a 65% yield of the free D-amino acid **61** after recrystallization. The optical purity of the resolved allylglycine isomers, as well as that of the commercial preparations, was checked by conversion to the camphanamide methyl esters and subsequent gas chromatographic (GC) analysis. These derivatives were prepared by a modification of the method of Arnold,[324] in which the amino acid was first treated with the (*S*)-camphanic acid chloride (**62**) in a mixture of toluene and carbonate buffer (pH 10), followed by acidic work-up and esterification with diazomethane. The resultant camphanamide methyl esters and the side product, methyl camphanoate[325] (**63**), were analyzed by GC without purification. Results indicated that the L-allylglycine obtained from the resolution was 99.5% ± 0.5% pure, and the commercial material was 99.4%±0.5% pure. The commercial and the resolved D-isomers were found to be enantiomerically pure within the detection limits of this method (estimated at ± 0.5%). Subsequent purification of the analysis mixtures gave the camphanamides[324] **64** and **65** in 48% and 55% yields, respectively (Scheme 11).

These enantiomerically pure allylglycines were then protected before use in the ene reaction. The carbobenzyloxy (Z) group was chosen to protect the amine as it can withstand Lewis acids. Treatment of **60** and **61** with benzyl chloroformate produces the Z-2-amino-4-pentenoates **66** and **67** in 83% and 90% yields, respectively. Subsequent

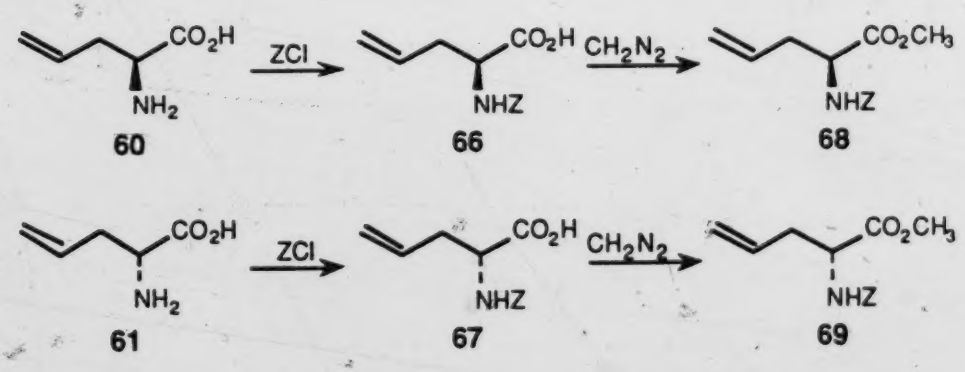
treatment with diazomethane gives the methyl esters **68** and **69** in yields of 96% and 83%, respectively (Scheme 12).

Scheme 11



With the requisite starting materials prepared , the ene reaction was attempted. Although a wide variety of Lewis acids can promote intra and intermolecular ene reactions, ferric chloride was initially chosen since it had been successful in the assembly of racemic DAP analogs.[60, 257] Initially two equivalents of the glyoxylate, one equivalent of the allylglycine and three equivalents of Lewis acid were combined for the ene reaction. However, these reagents led to an intensely fluorescent spot on TLC and the NMR spectrum of the reddish product was very broad, suggesting the presence of iron. Treatment of a portion of this compound with hydrogen sulfide failed to produce a precipitate or remove the reddish color. Attempts made to purify and characterize this compound further were unsuccessful, due to its instability under a variety of purification conditions.

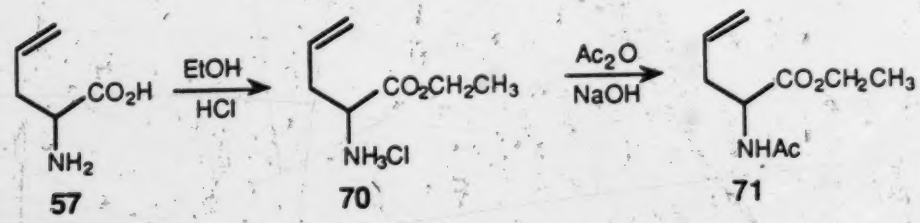
Scheme 12



To determine whether the Z group of the allylglycine was responsible for this strange behaviour, ethyl *N*-acetyl-allylglycine (**71**), which had been used in the ene reactions of Girodeau,[60, 257] was prepared. Racemic allylglycine was converted to the ethyl ester hydrochloride **70** in 88% yield, and then acetylated with acetic anhydride in base to give the protected allylglycine **71** in 67% yield (Scheme 13). When the reaction was repeated using **71** as the ene component, it was noted that the fluorescent spot on TLC

appeared when the glyoxylate was stirred with the ferric chloride and the allylglycine did not appear to react. Again attempts to purify and characterize this compound were unsuccessful. A possible explanation for this could be formation of a coordination complex of the iron with the glyoxylate in its hydrated form, although this cannot be confirmed with the evidence available.

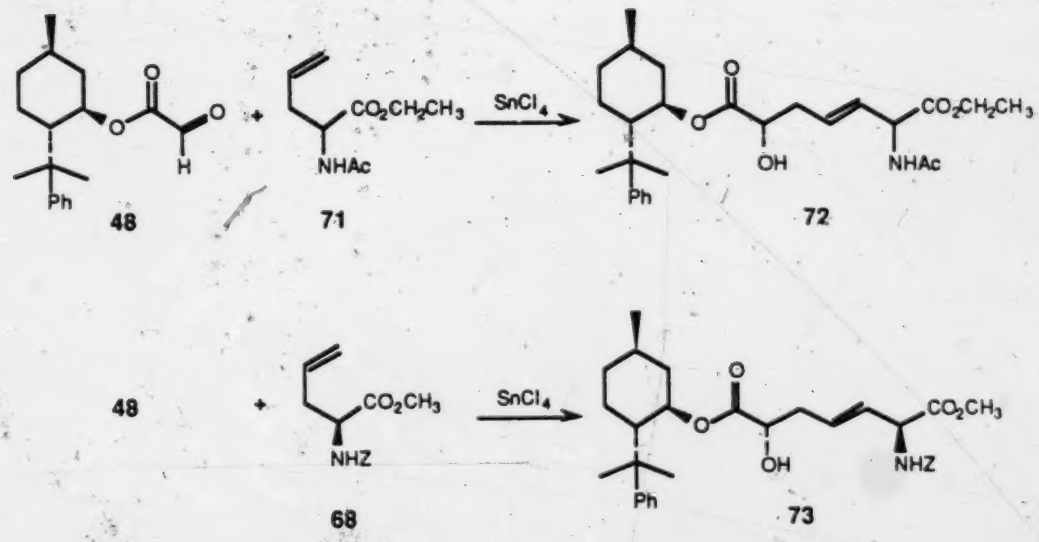
Scheme 13



57 **70** **71**

Since ferric chloride is an unsuitable catalyst for this ene reaction, stannic chloride, which had been successfully used in Whitesell's work with 8-phenylmenthyl glyoxylate (48), was chosen. The protected allylglycine presents several sites for Lewis acid complexation and thus more than one equivalent was used in the presence of a slight excess of the alkene. With these modifications, the ene reaction between the glyoxylate **48** and the racemic protected allylglycine **71** was again attempted. This time the homoallylic alcohol **72** was successfully produced in 57% yield. Encouraged by this result, the ene reaction employing the Z-protected allylglycine ester **68** was also tried with similar success, yielding 62% of the condensation product **73** (Scheme 14).

Scheme 14

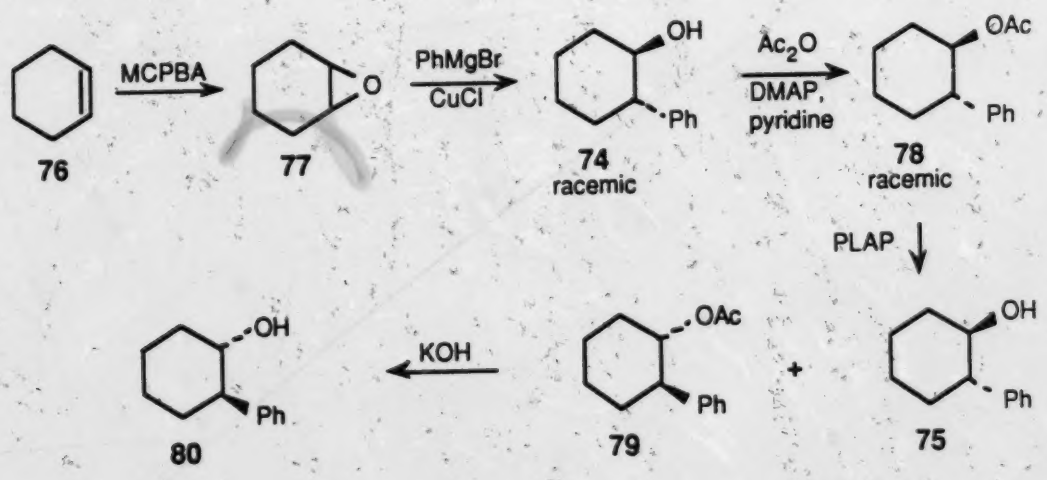


Because the pulegone used to prepare the (-)-8-phenylmenthol chiral auxiliary was optically impure (96% e.e.) and relatively lengthy procedures were required to prepare the enantiomer , a different chiral auxiliary, *trans*-2-phenylcyclohexanol (**74**), which was also developed by Whitesell and coworkers,.[251, 326, 327] was chosen as a replacement. This chiral alcohol is easier to prepare than the (-)-8-phenylmenthol and both isomers are readily available by enzymatic resolution. This chiral auxiliary has been used in several different reactions,[256, 265, 272, 280, 285, 308, 328-332] including ene reactions,[256, 272, 326, 333, 334] where it has given diastereomeric ratios of 95:5, slightly lower than those of the 8-phenylmenthol. Furthermore, (1*R*, 2*S*)-*trans*-2-phenylcyclohexanol (**75**) is known to induce the opposite stereochemistry to (-)-8-phenylmenthol (**53**) in ene reactions with the glyoxylates, although the spatial relationships of the phenyl groups are the same.[256, 334] Although the reason for this reversal is not clear, Whitesell has proposed that this is due to different roles of the phenyl groups in each of the compounds. In 8-phenylmenthyl glyoxylate (**48**) there is the possibility that the phenyl group may act as a

π-donor.[251, 254, 256] This interaction seems less likely with the glyoxylate derived from *trans*-2-phenylcyclohexanol (**75**), because of the distance of the phenyl group from the glyoxylate carbonyls.[256]

To prepare this auxiliary, the method of Whitesell[327] was utilized (Scheme 15). Cyclohexene (**76**) was oxidized in the presence of *meta*-chloroperbenzoic acid (MCPBA) to give cyclohexene oxide (**77**) in 60% yield. The epoxide, which is also commercially available, was opened with phenylmagnesium bromide in the presence of copper chloride to give racemic *trans*-2-phenylcyclohexanol (**74**) in 79% yield. Of the several methods now available for the resolution of this alcohol,[327, 335, 336] the method of Whitesell was chosen.[327]

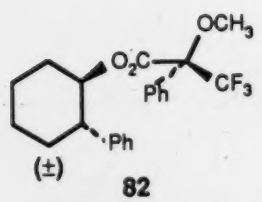
Scheme 15



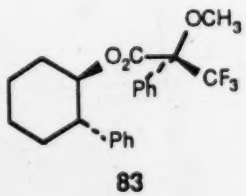
Alcohol **74** was acetylated to afford the acetate **78** in 94% yield. This compound was then incubated with pig liver acetone powder (PLAP) in the presence of acetone and phosphate buffer. To monitor this reaction a more facile NMR procedure was used, rather than the HPLC method suggested in the literature. This analysis relies on the difference in the ^{1}H NMR chemical shift between the proton adjacent to the hydroxyl relative to that of

the corresponding acetate. Integration and calculation of the ratio of these two peaks indicates the extent of reaction. The resolution was also performed using purified pig liver esterase (PLE) on smaller scale, but there was no advantage found over PLAP. Work-up and successive recrystallizations gave 56% of the optically pure alcohol **75**. The remaining acetate **79** was hydrolyzed in aqueous base, and the product was recrystallized to give optically pure alcohol **80** in 45% yield.

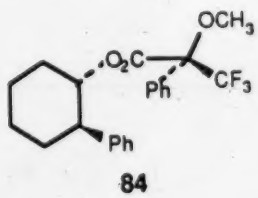
The optical purity of these alcohols was confirmed by conversion to their (R)-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA) esters and examination of the crude materials by ^{1}H NMR. Reaction of MTPA with oxalyl chloride prepared the α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) (**81**). This reacts with alcohols **74**, **75**, and **80** to produce the esters **82**, **83**, and **84** in 16%, 59% and 78% yields, respectively, after purification. The spectra indicated that the resolved *trans*-2-phenylcyclohexanols were enantiomerically pure within ^{1}H NMR detection limits (±1%).



82



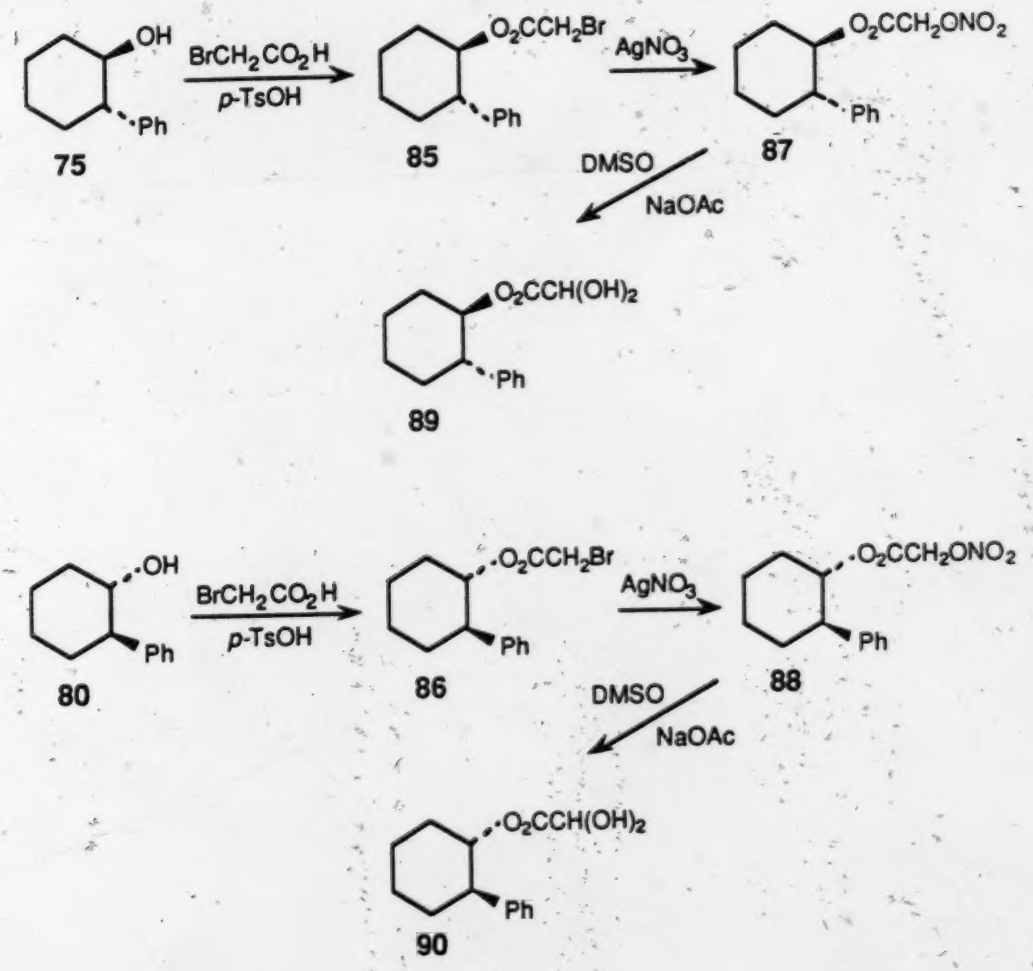
83



84

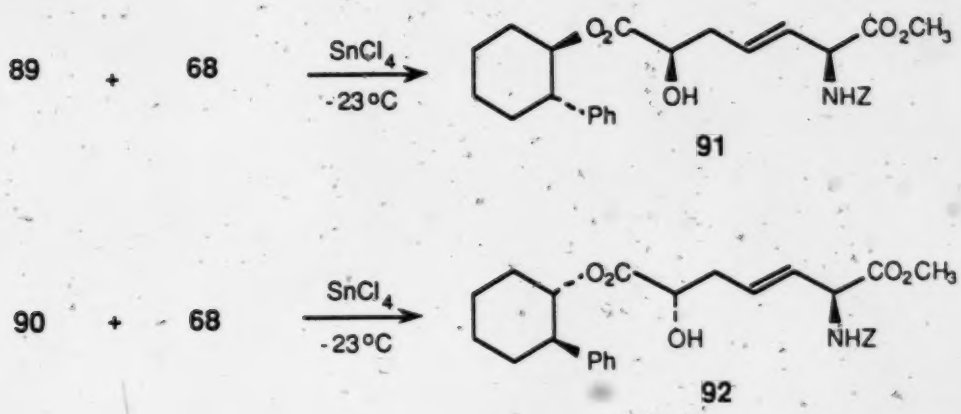
These alcohols **75** and **80** were converted the corresponding glyoxylates by literature procedures.[251] Esterification with bromoacetic acid and *p*-toluenesulfonic acid produced the bromoacetates **85** and **86** in nearly quantitative yields. Conversion of the bromoacetates to the respective nitrate esters **87** and **88** in 99% and 98% yields, respectively, was achieved by reaction with silver nitrate. Treatment with DMSO and sodium acetate gave the glyoxylate hydrates **89** and **90** in 94% and 92% yields, respectively (Scheme 16).

Scheme 16



The ene reactions between the hydrates **89** or **90** and allylglycine **68** were performed with at least four equivalents of Lewis acid: one to dehydrate the glyoxylate, one to complex with the product alcohol, and the remaining two to complex to the other sites on the allylglycine derivative. In this manner the homoallylic alcohol **91** was prepared in 83% yield. Similarly the diastereomer **92** was produced from **90** and **68** in 81% yield (Scheme 17).

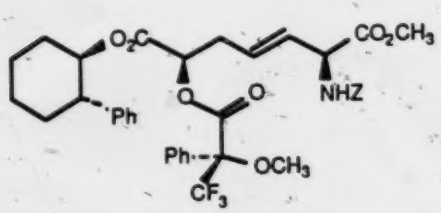
Scheme 17



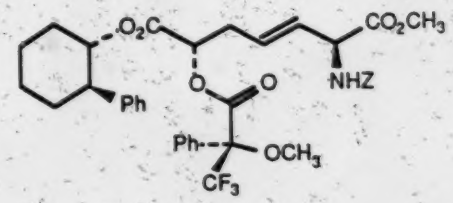
With the key alcohols **91** and **92** available, it was necessary to determine if any epimerization at the α-amino center had occurred and to examine the degree of asymmetric induction at the newly formed α-hydroxy center. Epimerization at the α-amino center of **91** will produce the enantiomer of **92** which will have the same NMR spectrum as **92**. Similarly the epimerization of **92** at the α-amino center will produce the enantiomer of **91**. Examination of a 2:1 mixture of **91** and **92** shows that there are visible differences in chemical shifts in the ^{13}C NMR spectra for the ester carbonyl, the quaternary aryl, the alcohol and one of the cyclohexyl methylene signals. The spectra demonstrate that there is no epimerization at the α-amino carbon within the detection limits of ^{13}C NMR spectrometry (± 5%).

To investigate the degree of asymmetric induction at the α-hydroxy position it was necessary to prepare the (R)-(+)-MTPA esters of **91** and **92**, as well as a 2:1 mixture of these two compounds. Attempts to prepare these derivatives by the previously described condensation with the acid chloride were unsuccessful, giving only trace amounts of the desired materials. Dicyclohexylcarbodiimide (DCC) coupling with the free acid also failed and Steglich esterification[337] in the presence of DCC and *N,N*-dimethylaminopyridine

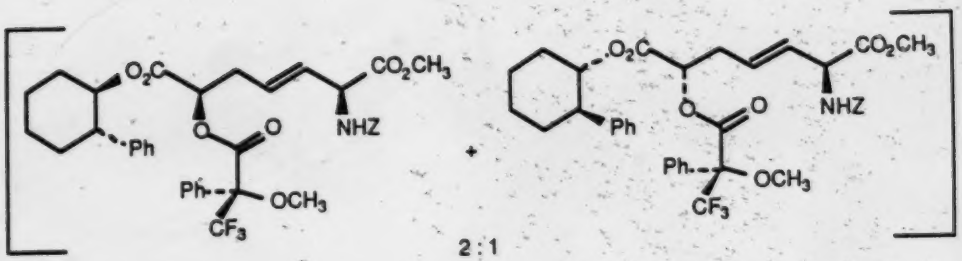
(DMAP) produced unsatisfactory results. The most successful procedure found was that of Boden and Keck[338] in which both DMAP and DMAP hydrochloride (DMAP·HCl) were added with the DCC and free MTPA acid. By this method, the MTPA esters **93**, **94**, and **95** were formed in purified yields of 37%, 53%, and 55%, respectively.



93



94



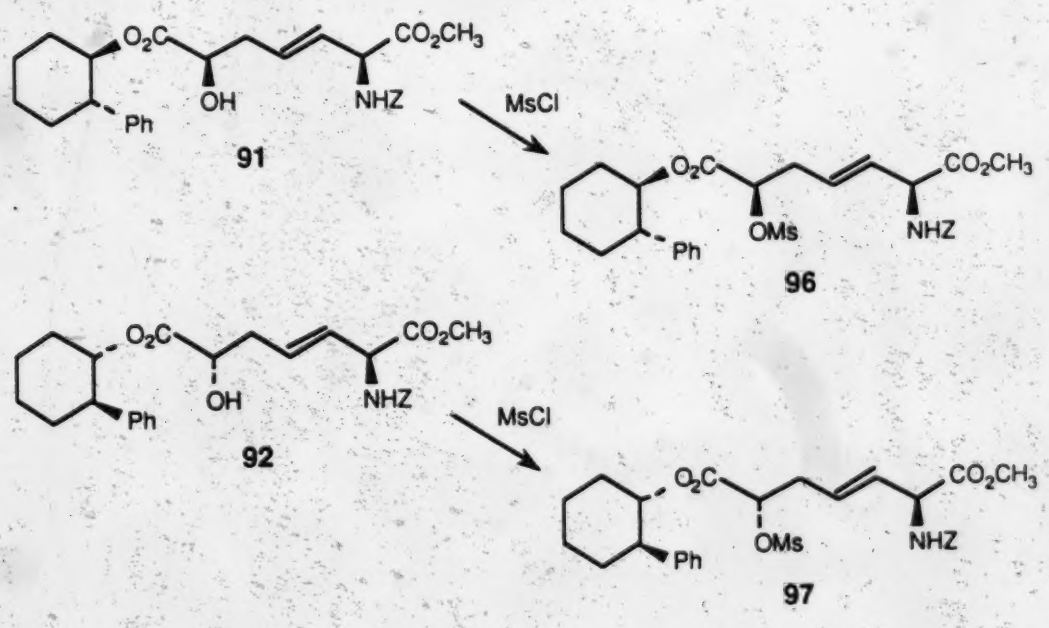
95

However, examination of the ^{1}H NMR spectra of these esters revealed three peaks for the *O*-methyl group as opposed to the one or, at most, two expected. Furthermore, the relative ratio of these peaks could be manipulated by changing the amount of DMAP and DMAP·HCl present. This indicated that epimerization was occurring during the esterification. Additional evidence for racemization was found when the alcohols **91** and **92** were exposed to DMAP and DMAP·HCl under the same conditions. Comparison of the ^{13}C NMR spectra of these materials with that of a 2:1 mixture of **91** : **92** showed these spectra to be almost identical. Hence the alcohols are racemized under the esterification conditions and the epimerization may be occuring at the α-amino center. However, there is insufficient evidence to confirm the position of epimerization. From the MTPA ester data it

could be determined that alcohol **91** was at least 77% one isomer, and alcohol **92** was at least 92% one isomer.

Of the several methods available to convert the hydroxyl group to an amine, the initial choice was conversion of the hydroxyl to a mesylate and subsequent displacement to form an azide which could then be easily reduced to the amine. This route was used successfully by Girodeau and coworkers in their synthesis of unsaturated DAP analogs,[60, 257] and more recently in a stereospecific synthesis of *allo*-isoleucine.[258] Alcohols **91** and **92** were treated with methanesulfonyl chloride (MsCl) in pyridine over two hours to give the mesylates **96** in 96% and **97** in 92% yields, respectively (Scheme 18).

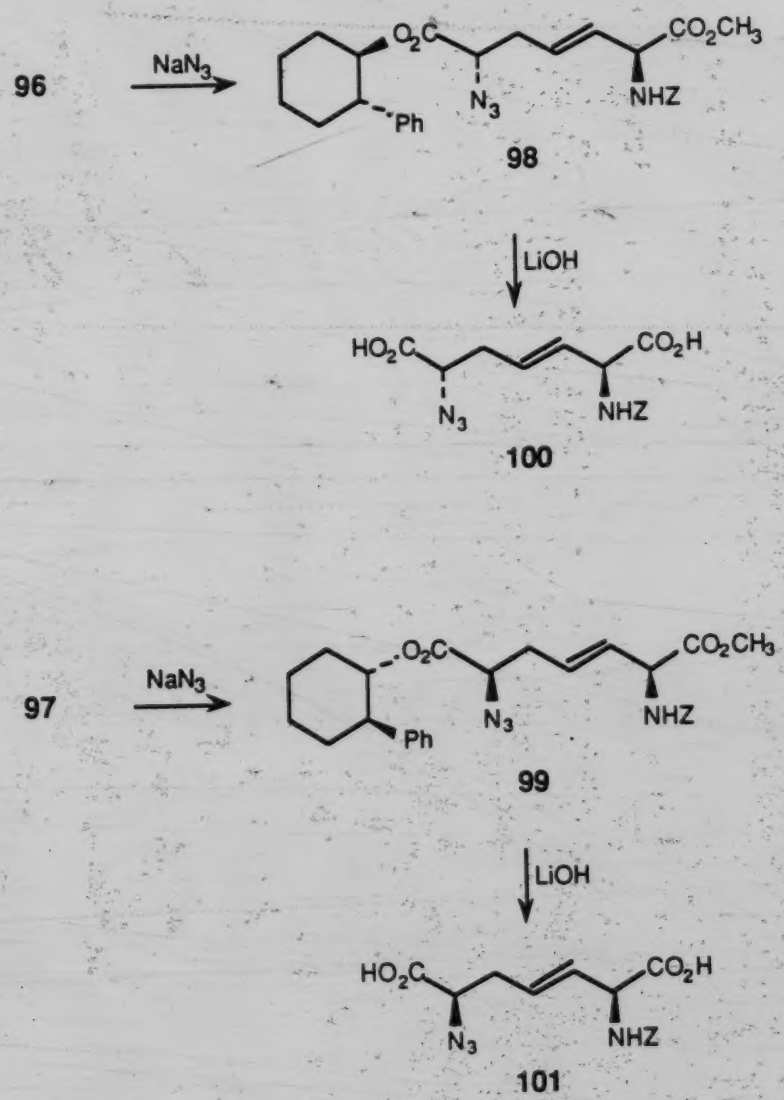
Scheme 18



The mesylates **96** and **97** were transformed into the unstable azides **98** and **99** in quantitative yield by treatment with sodium azide in dimethylformamide (DMF). Due to the

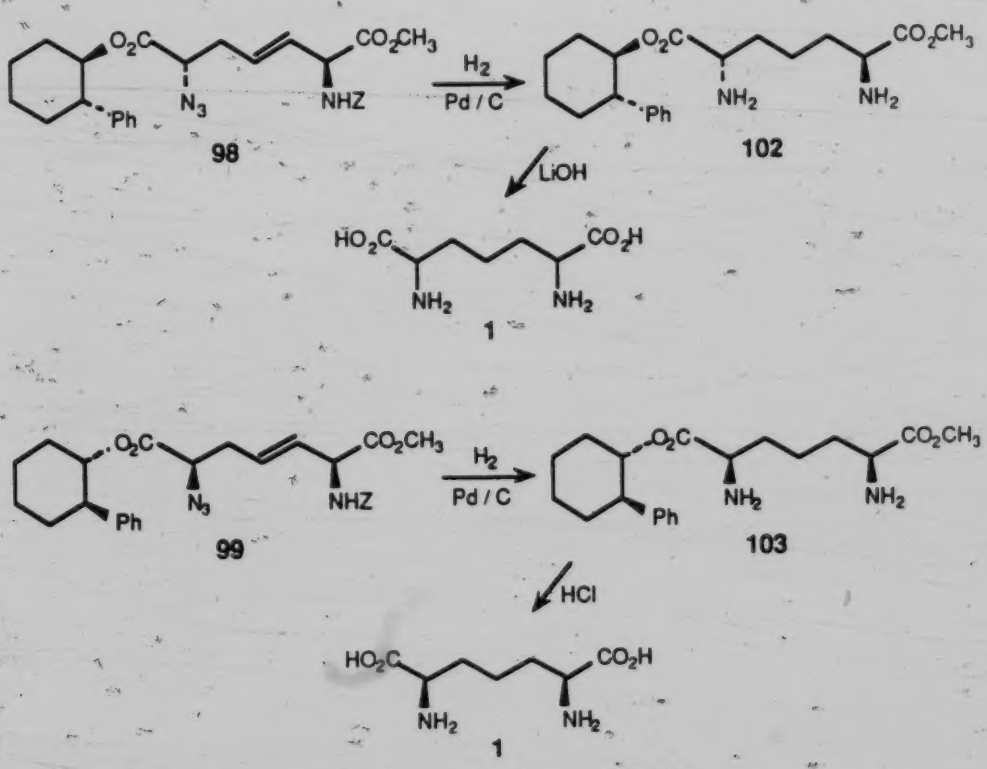
instability of these azides, the materials were immediately carried on to the next step. Initially, hydrolysis of the esters in base was attempted, to give the free acids **100** and **101** in yields of 80% and 85%, respectively, but these compounds were also very unstable and could not be fully characterized (Scheme 19).

Scheme 19



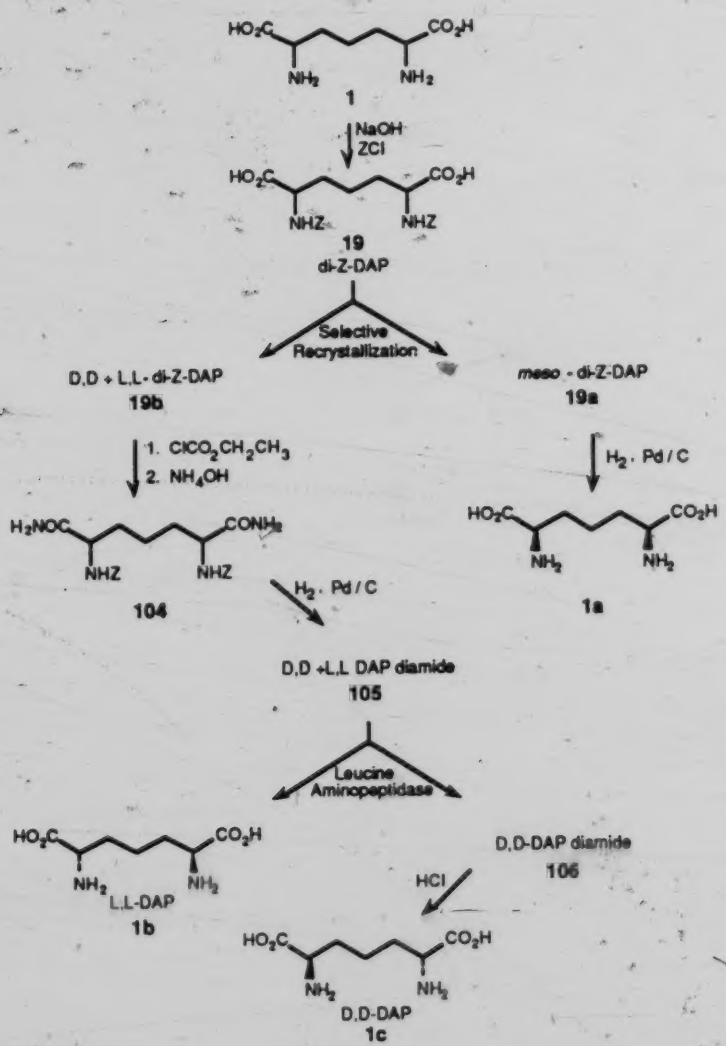
Because the basic hydrolysis failed to give stable materials, the fully-protected azides **98** and **99** were subjected to hydrogenation in acetic acid (AcOH) immediately after preparation, to quantitatively produce diamines **102** and **103** as their diacetate salts. The hydrogenations were also performed in MeOH with two equivalents of AcOH, to give diamines **102** and **103** in 29% and 49% yields, respectively, after partial purification by ion exchange chromatography. These compounds proved very difficult to purify fully and were carried on to the final hydrolysis without further purification. Conversion of the diamine **102** to DAP (**1**) was accomplished in 82% yield by hydrolysis with lithium hydroxide in tetrahydrofuran (THF) and water. Alternatively, the esters of **103** were cleaved by heating to reflux in 5.7M HCl to afford DAP (**1**) in 96% yield (Scheme 20).

Scheme 20



The previous success in determining optical purity by GC analysis of camphanamide methyl esters of amino acids[324] suggested that a similar approach could be used on the bis-camphanamide dimethyl esters of DAP. To develop this procedure, pure samples of each DAP isomer were required. *meso*-DAP (**1a**) and D,D-DAP (**1c**) had previously been resolved in our research group by Dr. Lee Arnold[324] using an approach similar to that shown in Figure 13.

Figure 13. Resolution of DAP isomers



To obtain the enantiomerically pure L,L-DAP (**1b**), a mixture of D,D- and L,L-di-Z-DAP **19b**, prepared previously by Mr William Sherwin, was converted to the di-Z diamide **104** in 79% yield by treatment with ethyl chloroformate and ammonia. This procedure was an improvement over that used previously.[324] Hydrogenation in acetic acid gave the diamide **105** as the diacetate hydrate in 99% yield. The diamide could then be resolved by the action of leucine aminopeptidase, which cleaved the L,L-diamide to the free amino acid **1b**. This latter reaction was performed by Dr. Lister Lam. Conversion of the pure DAP isomers **1a**, **1b**, and **1c** and a sample of the commercial mixture (all possible isomers) to the desired bis-camphanamide dimethyl esters was achieved by slight modification of the method used for the allylglycine derivatives **64** and **65**. This produced compounds **107**, **108**, **109**, and **110** in yields of 88%, 85%, 87%, and 99%, respectively (Scheme 21).

Attempts to analyze the optical purity of these compounds by GC using the previous conditions proved unsuccessful because the derivatives of DAP are insufficiently volatile. However, examination of the high field ^{1}H NMR spectra revealed that the methyl ester signals were almost completely resolved and the relative amounts of each isomer could be determined to within ± 2% (Figure 14).

Scheme 21

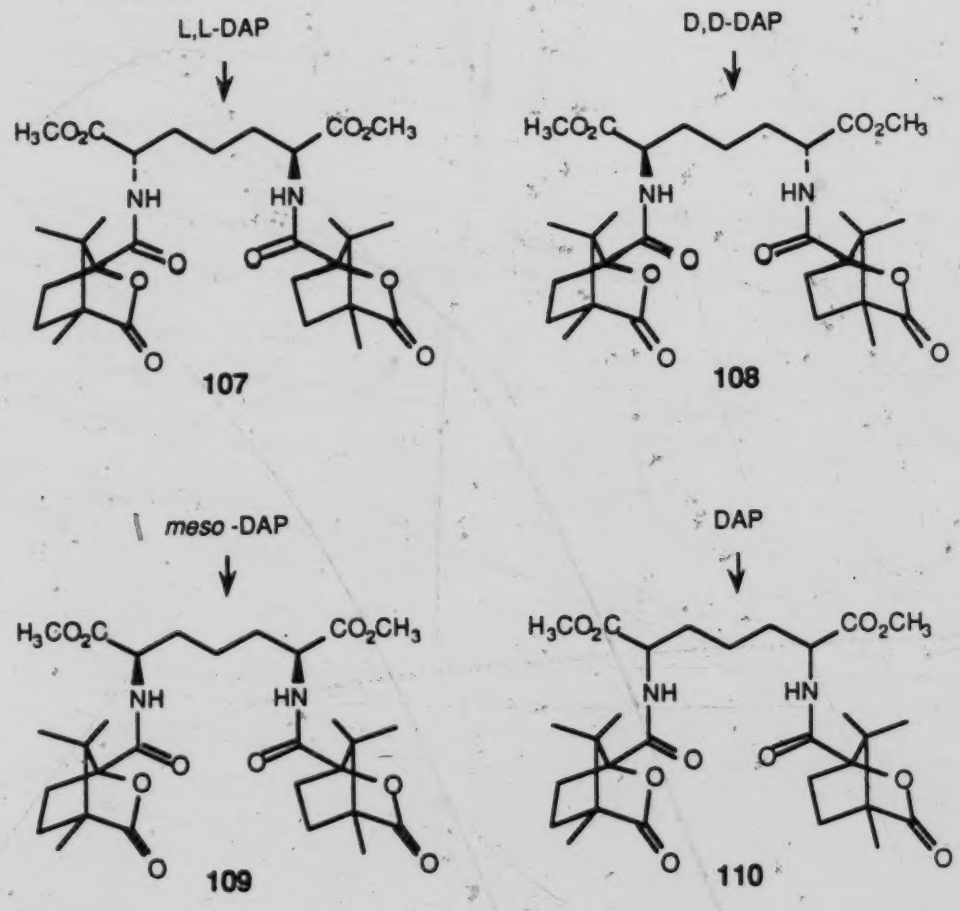
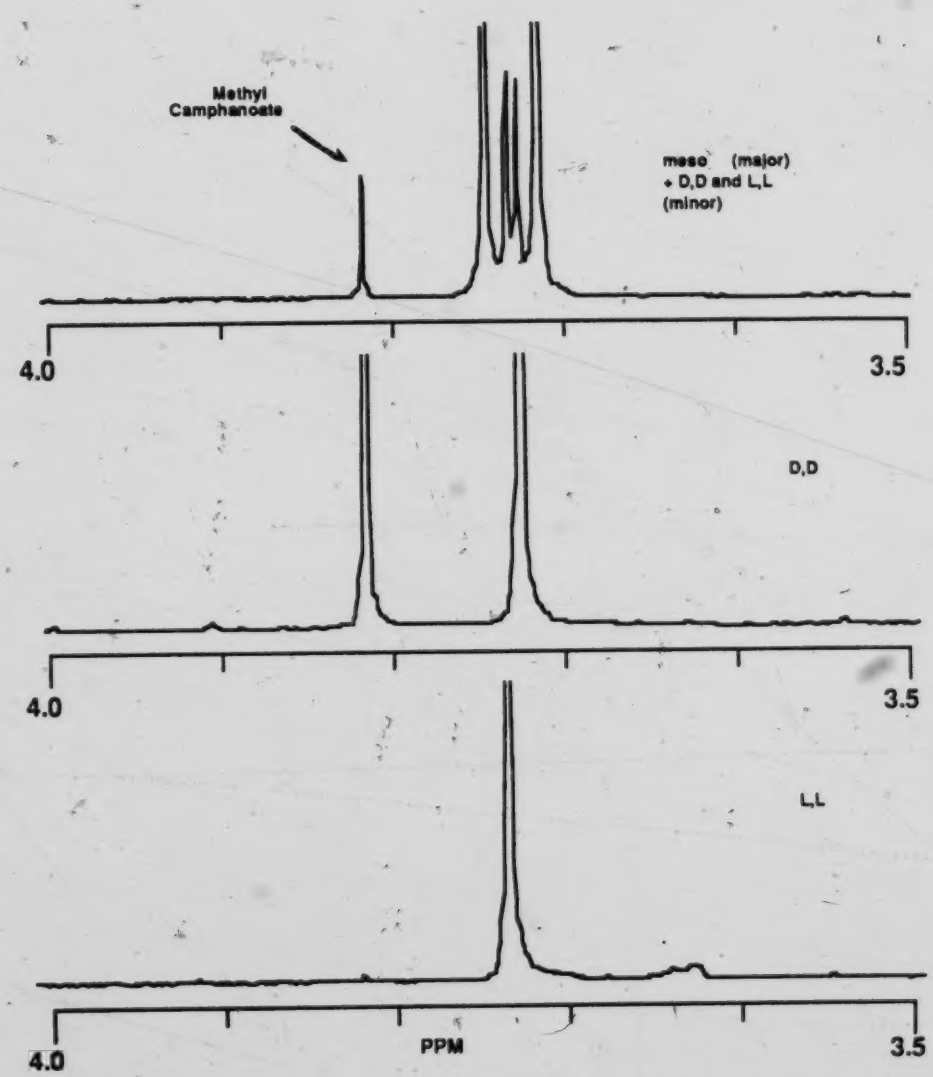


Figure 14. Determination of DAP Stereochemistry by ^1H NMR of the Methyl Ester Region of Camphanamide Derivatives



With a method for determining the stereochemistry of the synthetic DAP available, a portion of the DAP generated by basic hydrolysis of **102**, which had been hydrogenated in AcOH, was derivatized and the proton NMR spectrum was obtained. Disappointingly, the spectrum indicated that the DAP was almost completely epimerized at both centers, showing peaks for all three isomers. It was necessary to determine where this

epimerization had occurred. Using the same reasoning that was employed for the alcohols **91** and **92**, the ^{13}C NMR spectra of the mesylates should show if epimerization had occurred at the α-amino center through visible differences in a spectrum containing both mesylates **96** and **97**. This spectrum indicated several differences in chemical shifts of the two mesylates, but the spectra of the individual isomers did not reveal any epimerization at the α-amino center.

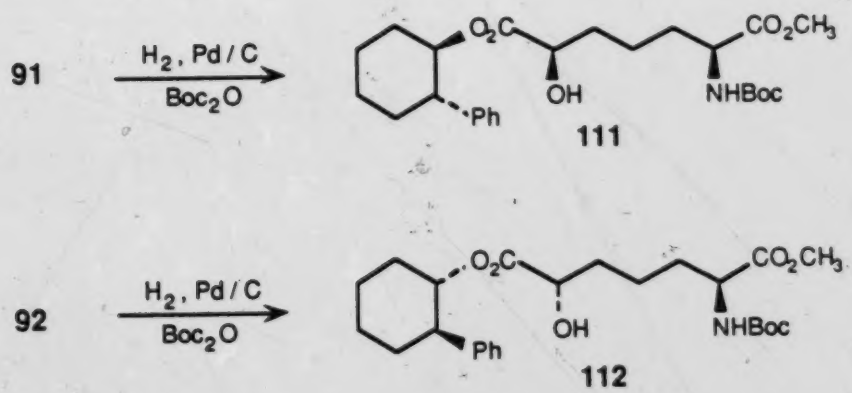
Basic ester hydrolysis in deuterated water should result in deuterium incorporation if epimerization is occurring during this reaction. Examination of both the proton and deuterium NMR spectra of the resulting DAP(96% yield), indicated no deuterium incorporation, and eliminated the possibility that epimerization occurs during ester hydrolysis. Similarly, the hydrogenation step was examined by using deuterated acetic acid as the solvent. Unfortunately it could not be determined with certainty whether epimerizaton had occurred in this reaction from the proton spectra obtained. This was due to the presence of impurities interfering with the signals of interest and the difficulties encountered in the purification of this compound. However, changing the hydrogenation solvent to methanol and adding only two equivalents of acetic acid to protonate the amines generated led to DAP which showed slightly less racemization, although all isomers were still present. DAP generated from diamine **103** hydrogenated under these conditions showed a considerable amount of D,D-DAP, which indicates that the epimerization is not confined to the α-azido center. The hydrogenation was also run in deuterated methanol and deuterated acetic acid, but again it could not be determined with certainty from the proton NMR spectrum that deuterium had been incorporated in the diamine produced.

These results indicated that the majority of the epimerization was occurring in the azide formation although some epimerization during hydrogenation was also apparent. Possibly the azide could be interacting with the internal double bond in some manner.

Removal of the double bond in the homoallylic alcohols **91** and **92** by hydrogenation could potentially prevent the epimerization occurring at the azide stage. The

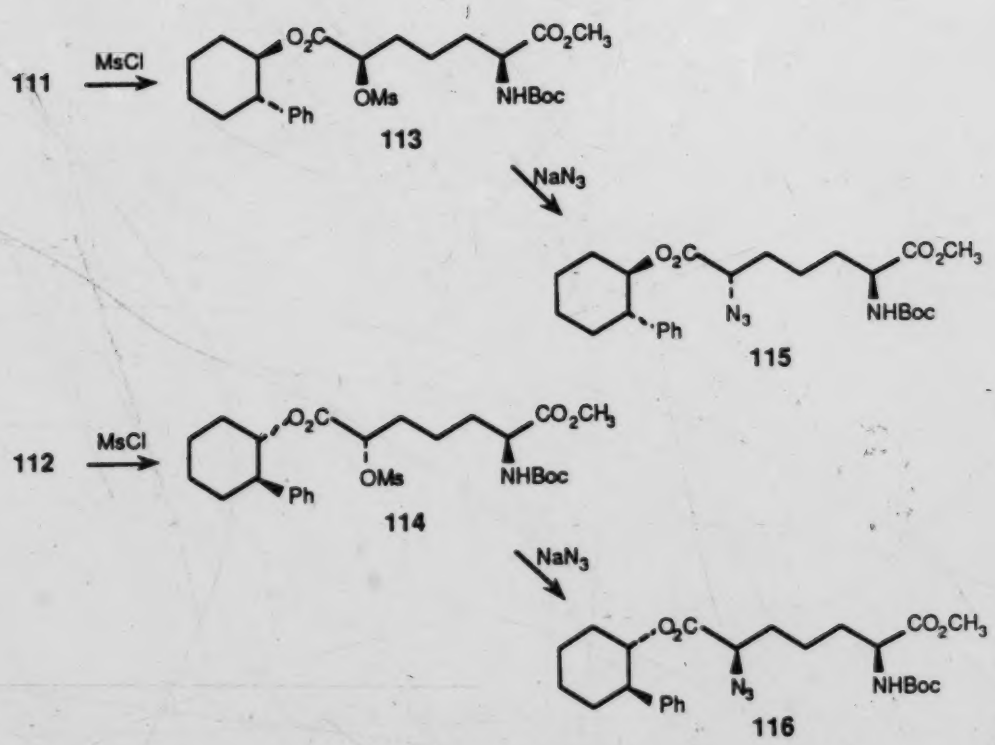
problem of the concomitant removal of the Z group in this reaction was solved by performing the hydrogenation in the presence of *t*-butoxypyrocarbonate (Boc_2O),[339] which reprotected the amine *in situ*. In this way, the Z-protected alcohols **91** and **92** were converted to the *t*-butoxycarbonyl (Boc)-protected compounds **111** and **112** in 84% and 80% yields, respectively (Scheme 22).

Scheme 22



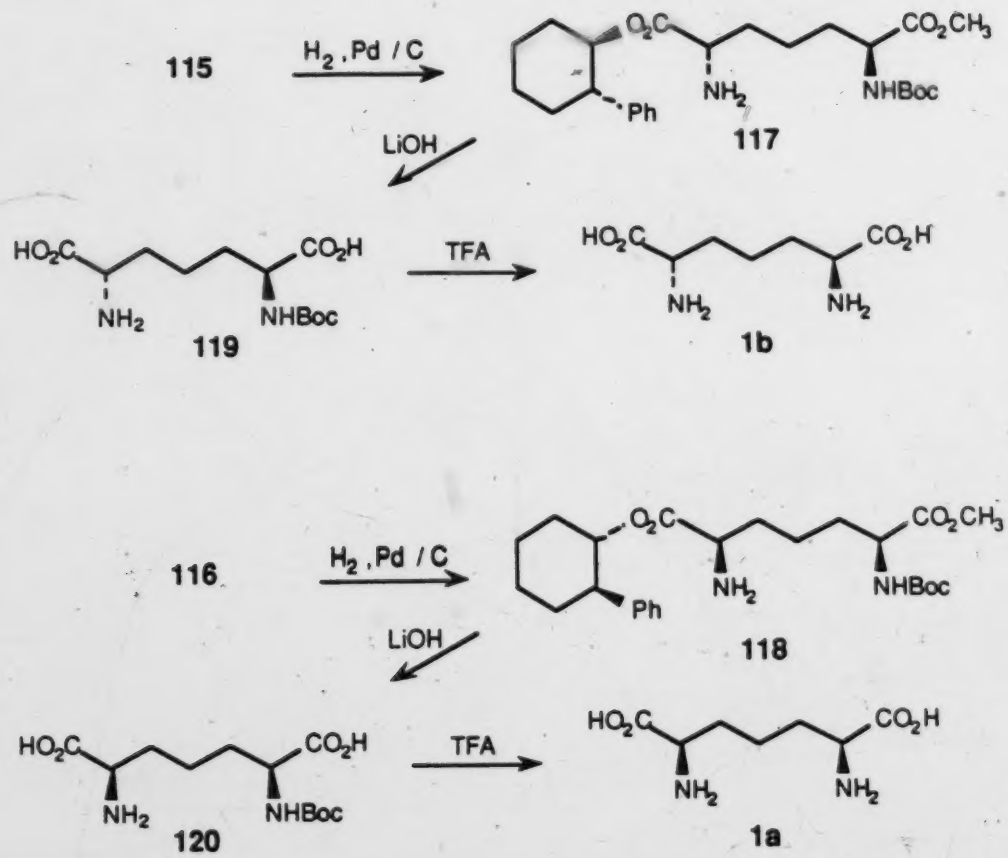
The Boc-alcohols **111** and **112** were converted to mesylates **113** and **114**, in 94% yield by a procedure analogous to that used for the Z-alcohols **91** and **92**. Treatment with sodium azide in DMF afforded azides **115** and **116** in 88% and 77% yields, respectively (Scheme 23). In contrast to the Z-azides **98** and **99**, the Boc-azides **115** and **116** were found to be quite stable. Thus it appears that the removal of the double bond does increase the stability of the compounds. An alternative route to the azide via a Mitsunobu reaction using diphenyl phosphoryl azide was also found to be successful, but there were difficulties removing excess azide reagent from the final product.

Scheme 23



Hydrogenation of the azides **115** and **116** in methanol produced the selectively protected DAP's **117** and **118** , in 80% and 93% yields, respectively. This material was carried through without further purification. Hydrolysis of the esters with excess lithium hydroxide gave diacids **119** and **120** (Scheme 24). The lithium chloride generated after a mild acid work-up in this reaction could not be easily removed from these compounds, so they were carried on without further purification. It should be noted that this hydrolysis failed to go to completion after several days when only two equivalents of base were employed.

Scheme 24



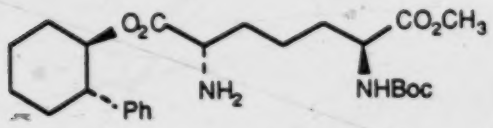
DAP was readily generated by treatment of the Boc-protected compounds **119** and **120** with TFA (Scheme 24). In these cases, the amino acid was not purified but directly derivatized to the bis-camphanamide dimethyl esters. Examination of the NMR spectra revealed a ratio of 84:16 (L,L: *meso*) for the DAP (**1b**) produced from **119**. Analogously, a ratio of 14:86 (L,L: *meso*) was found for the DAP (**1a**) derived from **120**. The desired amino acid was also generated by direct hydrolysis of the Boc diester **118** in HCl to give a 73% yield of (**1a**). This DAP sample was also derivatized and the proton NMR spectrum examined. A ratio of 16:84 (L,L: *meso*) was observed, in agreement with that found for the DAP (**1a**) generated by basic hydrolysis and TFA treatment.

To determine where this racemization may have occurred several approaches were investigated. As in the case of the Z-alcohols, epimerization of the α-amino center in **111** and **112** will produce the enantiomer of compound **112** and **111**, respectively. The ^{13}C NMR spectra of a mixture of the two alcohol isomers indicated sufficient differences in chemical shifts for the quaternary aryl, the alcohol, and the central methylene of the backbone, such that this method could reliably reveal epimerization at the α-amino center. Examination of these spectra for the alcohols **111** and **112** indicated no observable epimerization. Both the mesylates **113** and **114**, as well as the azides **115** and **116**, were also examined in this way. The ^{13}C NMR spectra of the mesylates showed some racemization. Estimation of the ratio of the two compounds by integration of the ^{13}C NMR peaks for the internal methylene of the chain indicated a ratio of 85:15 for **113** and a ratio of 88:12 for **114**. To test whether the epimerization was occurring at the α-amino center, the alcohols **111** and **112** were stirred for several days in dry pyridine under the conditions used for the mesylate reaction and no observable epimerization was detected. Mesylate **114** was also stirred for three days in dry pyridine. No difference was found between the ratios of this material and the original mesylate. This suggests that racemization has occurred at the α-hydroxy center before mesylate formation and is only visible on esterification, and that the mesylate center is not epimerized under the conditions. This view is substantiated by the fact that no detectable D,D material is seen in the DAP camphanamide spectra for the amino acid generated from both **118** and **120**. D,D DAP would arise only by epimerization of the α-amino center of these precursors for *meso*-DAP (**1a**). Mesylate formation has been claimed to proceed without epimerization.[258] However, the conditions used for the mesylate preparation in this latter work were different from those used here.

The differences in chemical shifts in the ^{13}C NMR spectra of the azides were small but visible, and an examination of the individual isomers revealed no apparent racemization. This may be due to the small peak of the other isomer being obscured by the

other very close peak or, more likely, that the epimerization at the α-azido center is not observable by this method. However, azide displacement of mesylates has been reported to proceed with complete inversion of configuration.[258, 340]

The use of ^{13}C NMR to probe potential epimerization at the α-amino center was unsuccessful in the investigation of the amines **117** and **118**. Here no observable differences were found in the ^{13}C NMR spectra of a mixture of the two isomers. Instead the hydrogenation was done in deuterated solvent with deuterium gas. No deuterium incorporation was detected in either of the α-positions as detected by proton or deuterium NMR.



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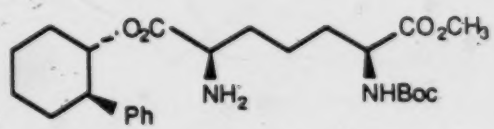
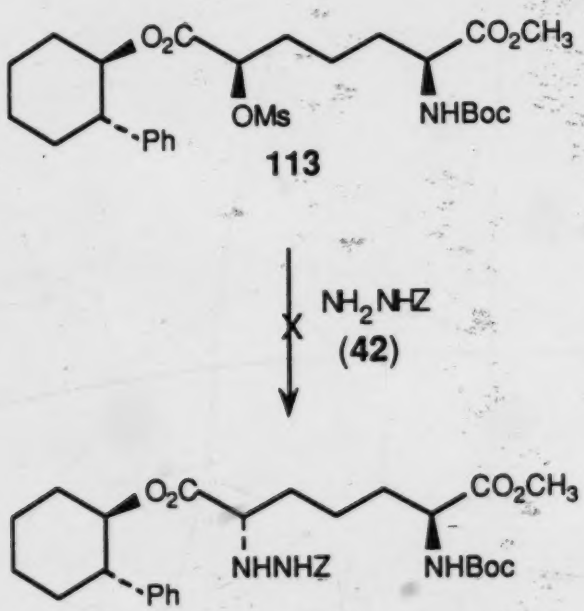


118

The lithium hydroxide hydrolysis of diesters **117** and **118** to compounds **119** and **120** was also examined for deuterium exchange, using deuterated solvent and lithium hydroxide hydrate which had been exchanged several times with deuterium oxide. Again, the deuterium NMR spectrum did not show any observable deuterium incorporation. Similarly, the acidic hydrolysis of compound **118** in 20% DCl in deuterium oxide failed to give any deuterium incorporation observable by proton NMR.

With these results and those found for the MTPA esters of the Z-alcohol, it appears that some epimerization of the α-hydroxy center has occurred. The most likely point of epimer formation appears to be in the original construction of the α-hydroxy center by the ene reaction. The absence of significant D,D-DAP in the amino acid generated from **118** and **120** as well as the ^{13}C NMR data for the alcohols **111**and**112** in pyridine provides evidence for epimerization of the α-hydroxy center rather than the α-amino center.

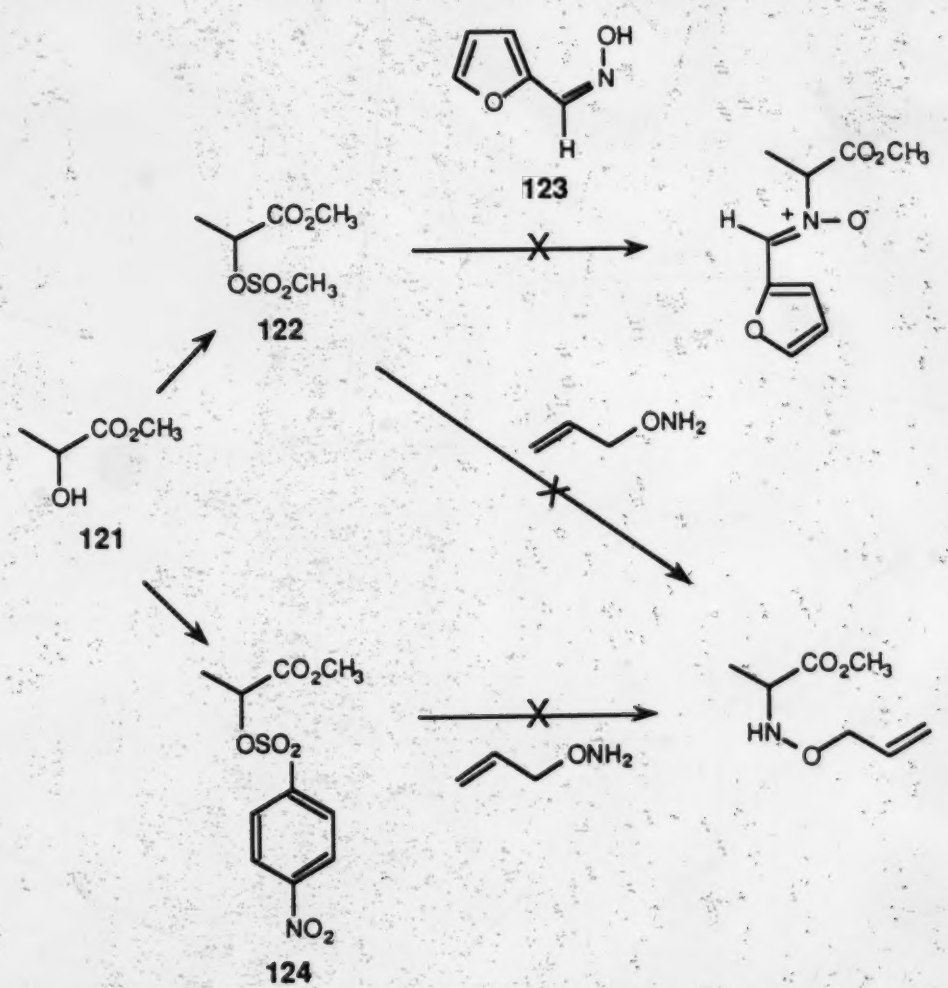
Expansion of this synthetic route to the preparation of the nitrogen-modified DAP analogs *N*-hydroxy DAP (**3**) and *N*-amino DAP (**4**) was then explored. In an attempt to prepare **4** from **113**, it was found that the mesylate group could not be displaced by the Z-carbazate **42**.



To determine whether the mesylate group was suitable for displacement with other nucleophiles such as protected hydroxylamines, a brief study was performed on the model mesylate derived from racemic methyl lactate (**121**). Methyl lactate (**121**), prepared by reaction of lactic acid with diazomethane in 90% yield, was treated with methanesulfonyl chloride in pyridine to give the mesylate **122** in 73% yield. This compound was then treated with *O*-allylhydroxylamine hydrochloride in the presence of base. The mesylate **122** was also condensed with *Z*-furfuraldehyde oxime (**123**), a derivative reported to be a good nucleophile for the generation of hydroxyamino acids.[341] This oxime was generated by reaction of furfural with hydroxylamine hydrochloride in the presence of base. Unfortunately, these nucleophiles failed to displace the mesylate group (Scheme 25). It

appeared that the mesylate was not a sufficiently good leaving group in the presence of nucleophiles weaker than azide.

Scheme 25



To test a better leaving group, the nosylate **124** of methyl lactate was also prepared in 56% yield from **121** (Scheme 25). However, reaction with *O*-allylhydroxylamine hydrochloride was again unsuccessful. Hence, preparation of a more active sulfonate,

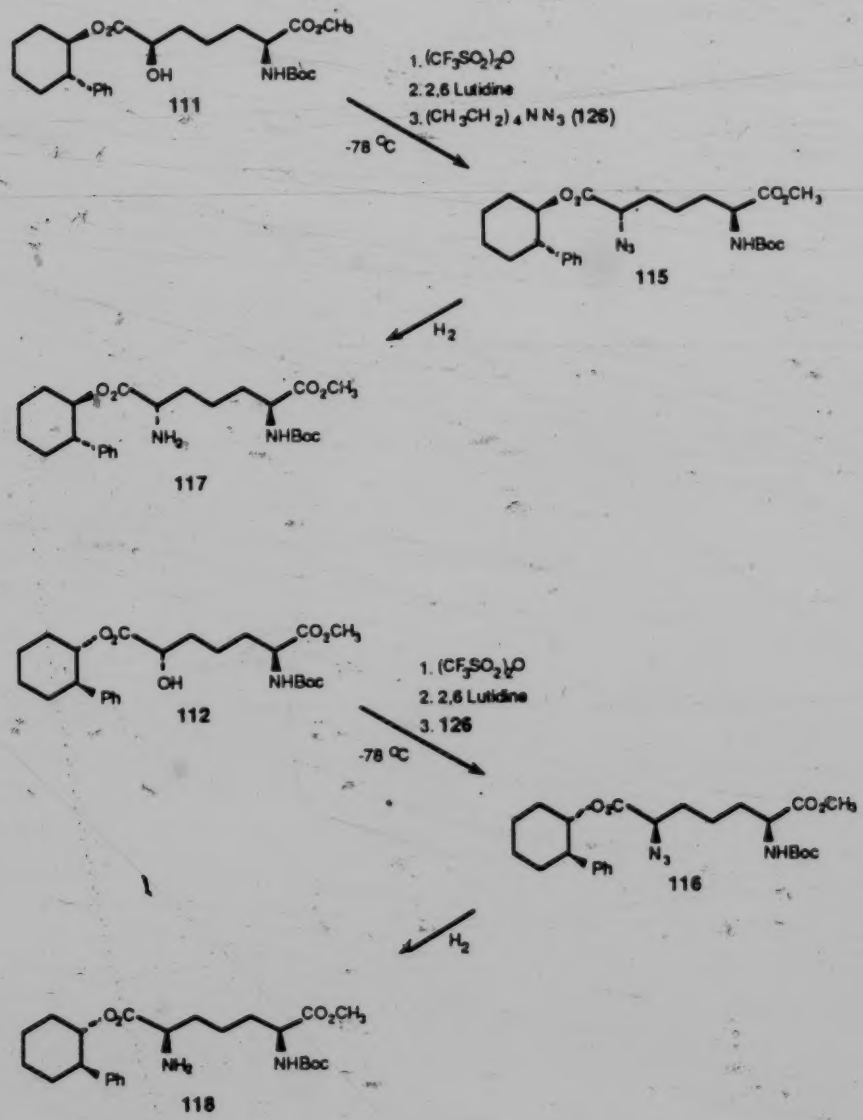
such as the triflate, appeared appropriate. Triflates have been successfully displaced by a number of nucleophiles to generate amino acids and nitrogen modified analogs.[181, 259, 342, 343] In addition, the triflates may be generated rapidly *in situ* at low temperatures without isolation prior to reaction with the nucleophile of choice, and this could reduce the amount of epimerization.

The reactivity of the triflic anhydride to nucleophiles necessitates the use of a non-nucleophilic solvent such as dichloromethane. This required the generation of an azide salt which was soluble in this solvent. Quaternary ammonium azides can be prepared easily by the reaction of hydrazoic acid with the appropriate quaternary ammonium hydroxide[344] and are soluble in organic solvents. The hydrazoic acid was generated by application of sodium azide to an ion exchange column in the protonated form. Neutralization of the eluent with tetramethylammonium hydroxide produced a poor (11%) yield of tetramethylammonium azide (**125**). However, by this method tetraethylammonium azide (**126**) was prepared in 83% yield. Using the procedure of Feenstra *et al.*[259], the triflate of alcohols **111** and **112** were prepared *in situ* and immediately treated with **126**. This afforded a 72% yield of the azide **115** and a 76% yield of azide **116**, respectively (Scheme 26).

Subsequent hydrogenation gave the amines **117** and **118** in quantitative and 90% yields, respectively (Scheme 26). HCl hydrolysis afforded the DAP isomers **1a** and **1b** in 83% and 72% yields, respectively. Derivatization to the biscamphanamide dimethyl esters and analysis of the proton NMR spectra indicated a ratio of 81:19 (*meso* : L,L + D,D) for **1a**. The presence of D,D DAP indicates some epimerization at the α-amino center in this case. From the camphanamide prepared from **1b** a ratio of 84:16 (L,L: *meso*) was determined. These results indicate that no appreciable decrease in epimerization is obtained in switching from the mesylate method to the triflate method. This suggests there is incomplete stereochemical induction in the original formation of the alcohols. However, Feenstra *et al.*[259] found a ratio of 88:12 in the preparation of some*N*-hydroxyamino acid

analogs using the triflate procedure, indicating that some epimerization could occur at this stage.

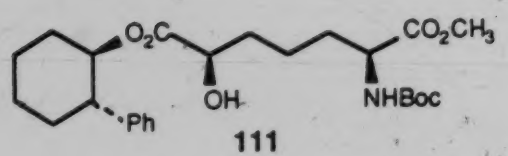
Scheme 26



Results from the analysis of the MTPA esters of the Z-alcohols also indicated incomplete induction of the α-hydroxy center. Thus the majority of the observed racemization of DAP is likely due to incomplete asymmetric induction in the ene reaction.

Displacement of the triflate by other nucleophiles was also attempted (Scheme 27).

Scheme 27



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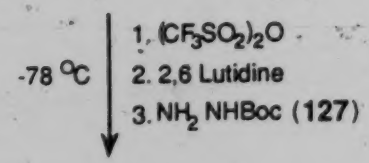

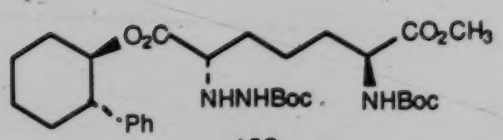

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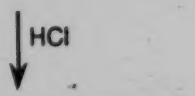

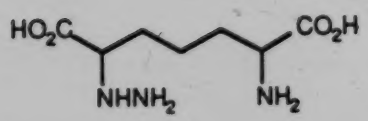

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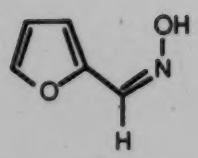

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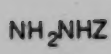

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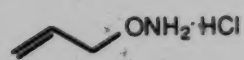
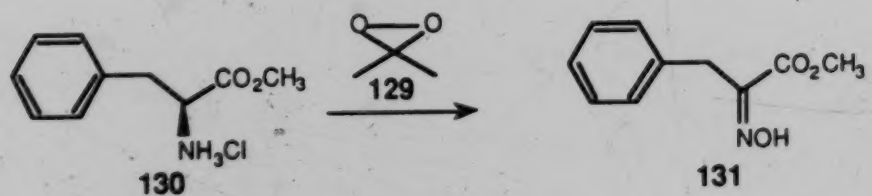


Treatment of the triflate generated *in situ* as before with Boc-carbazate (**127**) afforded a 61% yield of the protected hydrazino DAP compound **128**. Analogously, the triflate was treated with Z-carbazate (**42**), *O*-allylhydroxylamine hydrochloride and furfural oxime (**123**). Unfortunately, in these cases the displacement did not lead to the desired materials or led to thermally unstable compounds which could not be fully characterized. The Boc-protected hydrazino compound could be hydrolyzed in refluxing HCl to *N*-amino DAP (**4**) in 81% yield (Scheme 27). The stereochemistry of this material is currently under investigation.

Because the displacement with protected *N*-hydroxy nucleophiles was unsuccessful, the oxidation approach to these analogs was reexamined. A recent report by Danishefsky[345] indicated that *N*-hydroxy amino acids could be generated by oxidation with dimethyl dioxirane (**129**). This reaction was tested on the phenylalanine methyl ester (**130**), prepared in quantitative yield as the hydrochloride salt. However, it was found that this reaction resulted in over-oxidation to the oxime **131** (Scheme 28). At this point the work was discontinued.

Scheme 28



In summary, a stereoselective synthesis for DAP and several selectively protected moieties has been achieved. This strategy shows potential for the generation of other DAP analogs by displacement with suitable nucleophiles. In addition, a rapid method for the

determination of the optical purity of DAP has been developed and this method may be expanded to other diacids as will be described.

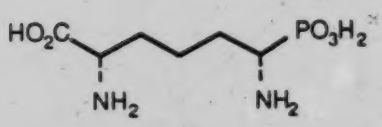
Another goal of this project was to test various DAP analogs as inhibitors and substrates for DAP epimerase (Figure 15). This information would provide greater insight into the enzyme specificity and the structure of the active site. The first set of analogs to be examined were the phosphono-DAPs **8-12**, prepared by Dr. Yonghong Song.

Before these compounds could be investigated, the epimerase had to be isolated and purified. Initially it was hoped to obtain enzyme of sufficient quantity and purity to crystallize for X-ray diffraction analysis. A modified isolation procedure based on that published by Higgins and coworkers[41] was followed, using the strain of *E. coli* JM109, harboring the plasmid pDF6,which contains the epimerase gene as the source of the enzyme. This strain was obtained as a generous gift from Dr. William Higgins, (Merrell-Dow Research Institute, Strasbourg, France) and cultures were grown by Dr. Michael Pickard in the Department of Microbiology, University of Alberta. It was found that the pellet obtained from the 30% ammonium sulfate fractionation contained a considerable amount of enzyme. This pellet was suspended and subjected to a second ammonium sulfate fractionation (0-15%. then 15-45%). The 15-45% pellet provided an enzyme solution of greater specific activity than that described by Higgins after DEAE cellulose chromatography. Hence, this column was omitted, and the enzyme was applied directly to a Matrex Green A (Amicon) dye-ligand affinity column. Following affinity chromatography the active fractions were concentrated by ultrafiltration (30,000 molecular weight cutoff).

The final purification in the published procedure involved fast protein liquid chromatography (FPLC). However, it was not possible to gain access to a FPLC apparatus with the appropriate column at the time and the scale of the isolation would have required numerous injections. Hence, additional purification was attempted using medium pressure chromatography on a short column of Q-Sepharose, an adsorbent similar to the

Figure 15. Substrate analogs to be examined with DAP epimerase

Replacement of carboxyl by phosphonate



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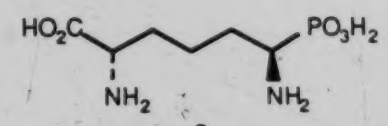

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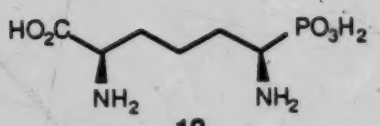

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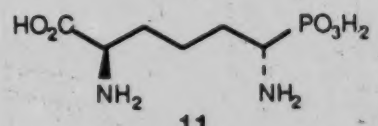

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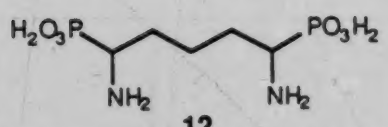

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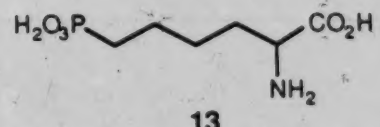
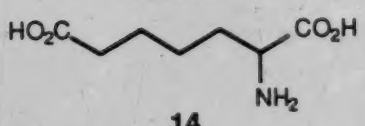


13

Removal of amine



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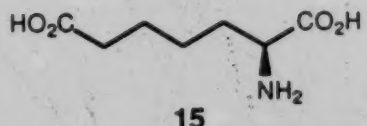
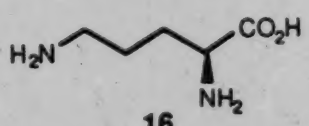


15

Removal of carboxyl



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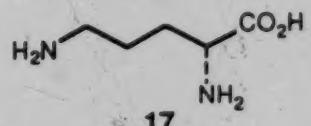
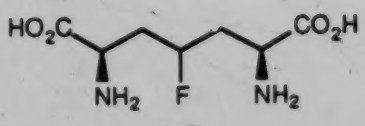


17

Substitution of chain



18

Mono-Q column used in the FPLC. This column was run with either a step gradient of 0, 0.20 M and 1.0 M NaCl in phosphate buffer or a continuous gradient between 0 and 0.20 M NaCl, followed by 1.0M NaCl, again in phosphate buffer. Examination of the active fractions by electrophoresis indicated that, under suitable conditions, this procedure resulted in considerable purification of the enzyme, although use of a larger column would be advantageous.

Unfortunately at this stage, the enzyme activity of the concentrated samples began to decrease rapidly. A number of tests were performed to determine the cause, and the results indicated that the storage conditions of the concentrated material were at fault. DAP epimerase contains an active site thiol known to be easily oxidized. Subsequent concentrated samples stored in 10 mM DTT buffer and tightly capped showed much less loss of activity. During these tests it was also determined that the addition of various protease inhibitors to the enzyme solution following ammonium sulfate fractionation preserved the activity relative to the untreated material. Phenylmethylsulfonyl fluoride (PMSF), which inhibits serine proteases, and pepstatin A, which inhibits acid proteases, were added singly or together to samples of the enzyme solution obtained from the 45% ammonium sulfate fractionation. The sample containing both PMSF and pepstatin A in concentrations of 7700 μM and 0.1 μM, respectively, retained the greatest activity over two weeks. The high protease activity in the crude samples obtained during ammonium sulfate fractionation had originally prevented the determination of a percentage recovery during this isolation procedure.

At this point the attempts to prepare DAP epimerase for crystallography purposes were abandoned and additional enzyme was purified to the stage of the affinity column following the literature procedure[41] with the exception of the concentration steps and the use of phosphate buffer instead of tris(hydroxymethyl)aminomethane (Tris) buffer. The epimerase generated had a specific activity of 15 units (U)/ mg and was pure enough to be used in the subsequent inhibition tests. Pure DAP epimerase has been reported to have a

specific activity of 192 U/ mg, where one unit of activity is defined as one μmol/min of NADPH formed under the conditions of the coupled enzyme assay.[41]

With the epimerase available, preliminary tests were performed on a mixture of phosphono-DAPs **8** and **9** (as a 4:1 mixture), and on the diphosphonate analog **12**, which was a mixture of all possible isomers. These compounds were initially examined using a coupled enzyme assay which employed DAP dehydrogenase.[40] The dehydrogenase enzyme used here had been previously isolated by Dr Greg Helms and Ms. Suzanne Crawley. In this coupled assay the conversion of L,L-DAP to *meso* -DAP by the epimerase is measured by further transformation of the *meso* -DAP to L-Δ^1-tetrahydrodipicolinate by DAP dehydrogenase with the concomitant reduction of nicotinamide adenine dinucleotide monophosphate (NADP) to NADPH. Results of these assays showed that the phosphono-DAP mixture **8** and **9** was a very weak mixed inhibitor for the epimerase (K_i= 24 mM , K_i'= 11.mM), while the diphosphonate was a weak mixed inhibitor (K_i= 4.2 mM, K_i'= 14 mM), as determined by the statistical method of Wilkinson.[346] Neither the phosphono-DAP mixture nor the diphosphono-DAP were substrates for the dehydrogenase nor did these compounds show any time-dependent inhibition of the epimerase in this study. However, a one point inhibition test of these compounds with the dehydrogenase showed that both the monophosphonate mixture **8** and **9** and the diphosphonate compound **12** inhibited the dehydrogenase about 18% and 10%, respectively, when the substrate (*meso* -DAP) concentration was 1mM in the presence of 1.8 mU enzyme.

When pure monophosphonate isomers became available, the compounds were initially tested against the dehydrogenase to determine whether the coupled enzyme assay could be used to examine the effect of these compounds against the epimerase.[55] This study was performed at pH 7.8, under the conditions at which the coupled enzyme assay would be run, instead of at the pH optimum of this enzyme (pH 10.5).[66] Under these conditions it was determined that none of the isomers **8-11** were substrates. However, significant substrate inhibition of the dehydrogenase was found at substrate (*meso* -DAP)

concentrations above 0.175 mM. This indicated that the inhibition of the epimerase by the phosphonate mixture **8** and **9**, and the diphosphonate **12** determined previously may be distorted. Subsequent examination of the individual phosphono-DAP isomers **8-11** revealed that all of these compounds weakly inhibited the DAP dehydrogenase (Table 2).

Table 2. Inhibition of DAP dehydrogenase by phosphonate analogs 8-11

Analog	Concentration (mM)	$K_m^{(I)}$ (mM)	K_i (mM)	$K_{i'}$ (mM)	Type of inhibition
8	2.25	0.12± 0.01	7.4	-	competitive
9	2.25	0.09± 0.02	-	12	noncompetitive
10	2.25	0.14± 0.01	4.3	-	competitive
11	2.25	0.10± 0.02	26	-	competitive

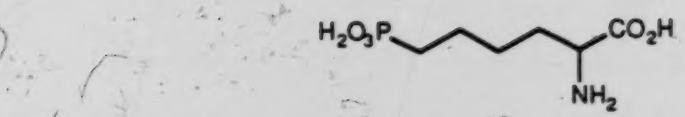
The monophosphonates **8-11** were also examined as potential time-dependent inhibitors of the dehydrogenase. No time-dependent inhibition was observed for any of the analogs when incubated with the enzyme at a concentration of 3.0 mM for up to 2 hours. This indicated that these compounds were not irreversible inactivators of the dehydrogenase.

The weak inhibition of the dehydrogenase by the monophosphonates **8-11** prevented the use of the coupled enzyme assay to examine the effects of these compounds on the epimerase. An alternative method available was a fixed-time one-point radioactive assay, in which the amount of tritium (^3H) released from generally labelled DAP was measured.[40] This method had not been expanded in the literature to obtain inhibition constants, thus this potential was investigated. The L,L-DAP concentrations chosen were based on those previously used for the coupled enzyme assay. Previous work on the radioactive assay by Dr. Lister Lam indicated that incubation of the radioactive DAP over 40 min would release 5% of the total radioactivity. Hence the specific activity of the

L,L-DAP solutions used was chosen so that, at the lowest concentration of substrate used, a sufficient number of counts would be generated to give an accurate value over background. This does not take into account the relative amounts of each tritiated DAP isomer in the labelled mixture.

The assay time was tested to ensure that the time of incubation did not result in complete depletion of the substrate at the lowest concentration. The enzyme (6.0 mU) was incubated in buffer containing 0.04 mM DAP for periods of 20, 30, 40, 50, and 90 min. Results indicated that the number of counts generated in the region of 20-50 min were directly proportional to incubation time, therefore the previously used time of 40 min was acceptable. It was also necessary to determine whether a K_m value comparable to that obtained by the coupled assay could be achieved. Data indicated that the K_m obtained by this method was the same as that generated by the coupled enzyme assay within experimental error. Hence, it appears that this radioactive method will be suitable for the examination of inhibitors with the epimerase.

In addition to the monophosphonates **8-11**, amino acids **13-18** were examined. L-Lysine (**16**) and D-lysine (**17**), which have been previously tested against the epimerase by other methods,[82, 347] were included as a further check of the procedure. Other amino acids were chosen because of their similarity to DAP, such as D,L- and L-α-aminopimelates **14** and **15**, and the phosphono-aminopimelate analog **13**. Finally, 4-fluoro-DAP **18** was briefly examined. Of these analogs, lysines (**16**) and (**17**), racemic α-aminopimelate (**14**) and its phosphonate analog **13** were commercially available, and the fluoro-DAP analog had been prepared by Dr. Yonghong Song. L-α-Aminopimelate (**15**) was resolved from the racemic material by literature procedures.[56] The optical purity of this compound was confirmed by preparing the camphanamide dimethyl ester **135** as previously described and comparing the proton NMR spectrum with those of the camphanamide dimethyl esters **136** and **137**. The latter were prepared from resolved D-α-aminopimelate (**133**) and the racemic material **14**, respectively.



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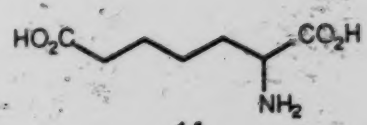

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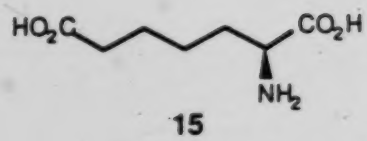

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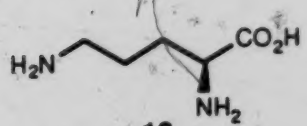

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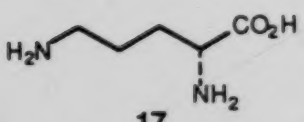

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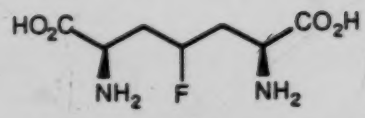

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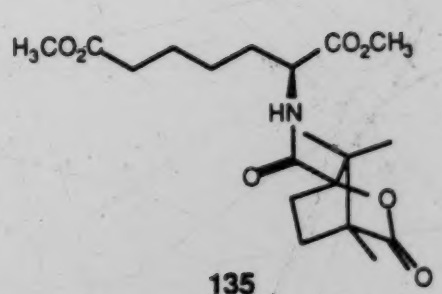

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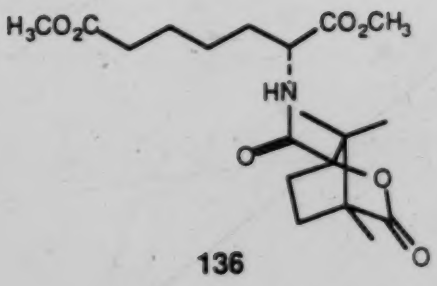

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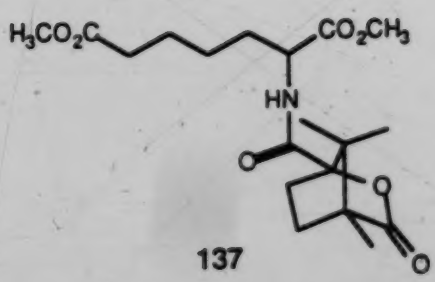
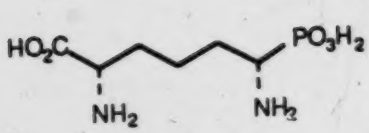


137

To test these inhibitors, the enzyme was assayed at various substrate (L,L-DAP) concentrations in the presence and absence of analogs. In the processing of this data it was

found that considerable variation occurred in the points derived from higher concentrations of substrate, most likely due to the volatility of the tritiated water produced. This in turn gave rise to fairly large errors in the apparent K_m's and V_{max}'s calculated using the statistical method of Wilkinson.[346] This is due to of the weighting given to the points obtained at higher concentrations in this method. To compensate for this, a change in the weighting of the various points was made. An individual standard deviation for the number of counts obtained at each concentration was calculated based on the variation found in the triplicate runs performed for each point. Recalculation of the results by this method, using a non-linear regression program and Michaelis-Menten kinetics, gave K_m values that were the same within experimental error to those calculated by the previous method but the errors were considerably reduced.

The results obtained for the inhibition of the epimerase are presented in Table 3. It was found that the *meso*-isomer **10** was not an inhibitor of the enzyme. Isomers **8**, **9**, and **11** were all found to be weak competitive inhibitors of the epimerase. A tenfold reduction in the concentration of inhibitor **8** in the assay relieved all inhibition. Initially an apparent increase in V_{max} was detected for both phosphonates **8** and **9**. To determine whether this was real or an artifact due to the high errors found in the assay at higher DAP concentrations, these two inhibitors were tested at high constant substrate concentration and the concentration of **8** and **9** in the assay was varied between 0 and 15 mM. The typical concentration of inhibitor used in these tests was 3 mM. For phosphonate **8** a negligible decrease in V_{max} was observed with increasing inhibitor concentration between 0 and 6 mM, and a more pronounced effect was observed in the 9 mM and 15 mM points. In the case of analog **9** a negligible effect on V_{max} was observed between 0 and 6 mM. However, a significant decrease was noted at 15 mM. The decreases noted at the higher concentrations may be partially due to concentration effects. The apparent increase in V_{max} caused by these two compounds is attributed to experimental error.



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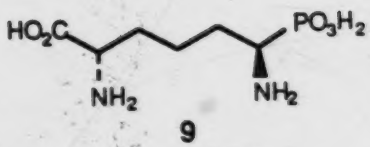

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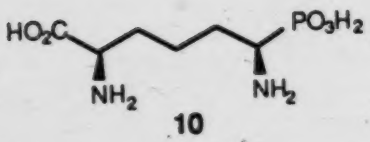

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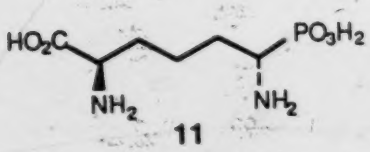


11

L-Lysine (**16**), did not inhibit the epimerase, and preliminary tests with D-lysine (**17**) indicated that this isomer also did not significantly inhibit the enzyme, in agreement with the previous literature report.[82] Similarly, the α-aminopimelate analogs **13** and **15** were found to be inactive. However, racemic α-aminopimelate (**14**) appeared to be a weak competitive inhibitor of the epimerase.

The fluoro-DAP analog **18** was studied by a one point inhibition test due to the small sample of this material available. It was found that this compound, tested at 3.0 mM, resulted in a large reduction in the amount of tritium released from the labelled substrate. However, considering the large concentration present, this could be a result of the material behaving as a substrate for the enzyme. Hence *meso*-DAP was tested under the same conditions. The results suggested that although a large amount of the decrease could be attributed to turnover of the analog, there was still 72% inhibition of the epimerase. No time-dependent inhibition was found when the enzyme was incubated with this inhibitor for up to two hours.

Table 3. Inhibition of DAP epimerase by analogs 8-17 using the radioactive assay

Analog	Concentration (mM)	$K_m^{(I)}$ (mM)	K_i (mM)	Type of inhibition
8	3.0, 0.30	0.55 ± 0.05	3.9	competitive
9	3.0	0.41 ± 0.06	9.3	competitive
10	3.0	0.33 ± 0.03	-	none
11	3.0	0.44 ± 0.02	7.2	competitive
13	3.0	0.25 ± 0.04	-	none
14	6.0	0.47 ± 0.04	5.8	competitive
15	3.0	0.32 ± 0.09	-	none
16	3.0	0.29 ± 0.02	-	none
17	3.0	0.18 ± 0.05	-	none

Each of the phosphonate analogs was also examined as a potential time-dependent inhibitor of the epimerase. There was no time-dependent inhibition of the enzyme observed when these analogs were individually incubated with the epimerase for up to two hours. Hence, none of these analogs is an irreversible inactivator of this protein.

To test the analogs **8-11** and **13-17** as substrates for the epimerase, each compound was incubated with the enzyme in deuterated buffer B, pD 7.4, for six hours. A control reaction was run simultaneously for each amino acid using heat inactivated enzyme. In addition DAP (**1**) (statistical mixture of all possible isomers) was also incubated under the same conditions with both active and inactive enzyme. The amino acid was subsequently isolated and examined for deuterium incorporation by proton and deuterium NMR. The DAP incubated with active enzyme revealed a 75% exchange of protons at the α-positions, and no deuterium incorporation was detected in that material which had been incubated with inactive enzyme. The 75% deuterium incorporation in DAP is expected, as D,D-DAP, which comprised 25% of the mixture, is not a substrate for the epimerase, in

contrast to *meso*- and L,L-DAP. This information also indicated that the enzyme reaction for its natural substrates had fully equilibrated during the time given. The complete lack of deuterium exchange into compounds **8-11** and **13-17** indicated that none of these analogs was a substrate for the epimerase under the conditions employed.

It is apparent from the above results that the monophosphonates **8-12** are relatively weak inhibitors for DAP dehydrogenase and DAP epimerase. The results determined for the inhibition of DAP dehydrogenase appear consistent with the rigid specificity found previously for this enzyme. Amino acids such as D- and L-lysine (**15**),(**16**), D,L-α-aminopimelate (**14**), and the glutamyl hydrazides **6** and **7** have been shown to be neither substrates nor inhibitors,[55, 66] indicating the requirement for both carboxyls and both amines in the natural substrate *meso* -DAP. The other isomers of DAP are not substrates but do behave as competitive inhibitors,[66] further demonstrating the strict specificity. Sulfur-containing DAP analogs such as *meso*-lanthionine, and analogs of this compound oxidized at sulfur, have been shown to be poor substrates, while the L,L and D,D isomers of these materials fail to be transformed.[55] However, previous results have shown that some modification of the nitrogen is permissible and that these materials can act as substrates, as discussed earlier. The strict specificity of the dehydrogenase is further demonstrated by the results obtained with the phosphonate analogs. It appears that the phosphonate group, which has been postulated to be a better binder in carboxylate binding sites,[204, 348] is too large to enter into the enzyme active site, and if it can, it cannot properly align itself for efficient binding. Hence, the compounds are neither substrates nor strong inhibitors.

The interaction of the various analogs with the epimerase provides a more complete picture of the features this enzyme requires in a substrate or inhibitor. It appears that, like the dehydrogenase, this protein also has a relatively strict substrate specificity and requires both carboxyls and both amines for effective binding of the substrate in the active site, as indicated by the failure of both the lysines (**16**) and (**17**), and the α-aminopimelates **14** and

15 to act as substrates or as good inhibitors. However, the weak inhibition exhibited by the racemic α-aminopimelate when the L-isomer was found to be inactive, suggests that the carboxyl group may be potentially more important than the amino group for binding at the distal site. This appears to be confirmed by the loss of inhibition on replacement of the carboxyl group by phosphonate in the analog **13**. Alternatively, this result may be interpreted as showing the inability of the phosphonate to fit into or align properly in the active site.

Potentially, the monophosphonates **8-11** could behave as substrates for the epimerase as, depending on the protonation state of the phosphonate, the phosphorus might be expected to enhance the acidity of the adjacent α-proton. However, none of the monophosphonates was found to be a substrate of this enzyme and this could again be due to the phosphonate failing to bind efficiently at the carboxylate site.

This failure to bind efficiently would also explain the weak inhibition of the enzyme by these materials. Literature reports indicated that substitution of a carboxylate by a phosphonate does not always result in increased binding. Instead, the correct alignment of functional groups within the enzyme active site and binding sites plays a more important role.[202, 204, 348] The tetrahedral phosphonate may not adequately align with the active site to be a good substrate, or cannot properly align with the distal binding site to bind to the enzyme. This could account for some of the apparently anomalous results found in the inhibition with the monophosphonates **8-11**. DAP epimerase is known to require an L-center at the distal binding site, because D,D-DAP is not a substrate or an inhibitor. Hence it would be expected that the monophosphonate **11**, which has a D-carboxyl site and a phosphonate site with the same relative configuration as a D-carboxyl site, would not be an inhibitor. However this compound is an inhibitor and it is the monophosphonate **10**, having the phosphonate in the same relative configuration as an L-carboxy site with a D-carboxyl site at the other end, which does not inhibit the enzyme. The inability of the tetrahedral phosphonate to properly align within the distal binding site when in the

"L"-configuration but to do so in the "D"-configuration compared to the normal planar L-carboxy group could explain these results. Inhibition of the epimerase by isomers **8** and **9** could be due to the L-carboxyl site binding at the distal site and the phosphonate being in the active site. In the case of isomer **8**, the phosphonate may also fit in the distal binding site. All of these results suggest that creation of DAP analogs by modification of the carboxyl groups does not produce effective inhibitors of DAP epimerase. Substitution on nitrogen as well as substitution along the chain, however appears to be acceptable. It would be interesting to see if an analog of DAP in which one nitrogen was replaced by a hydroxyl would be an effective inhibitor of this material.

Experimental

General

All reactions requiring anhydrous conditions were performed under a positive pressure of Argon using oven-dried (>12 h, 120 °C) or flame-dried glassware which had been cooled under Argon. The removal of solvent *in vacuo* refers to evaporation on a rotary evaporator followed by evacuation (<0.1 mm Hg) to constant sample weight. All solid products were dried *in vacuo* . Solvents for anhydrous reactions were dried according to Perrin *et al.*[349] Benzene, tetrahydrofuran (THF), and toluene were distilled from sodium and benzophenone. Triethylamine, diisopropylamine, pyridine, 2,6-lutidine, and dichloromethane were distilled from calcium hydride. Carbon tetrachloride and chloroform were distilled from P_2O_5. Anhydrous ethyl alcohol and methyl alcohol were prepared by distillation from magnesium metal and catalytic iodine. Solvents used for chromatography were distilled. Water used was Milli-Q (Millipore) quality, degassed *in vacuo* when necessary. Aqueous hydrochloric acid (HCl) was prepared free of metal ions from Milli-Q quality water and distilled as constant boiling (*c.a.* 110 °C) 5.7M HCl.

All reagents used were of ACS grade or finer. Air sensitive reagents were handled under an atmosphere of Argon. Diazomethane (CH_2N_2) was prepared using Diazald™. All commercial organometallic reagents were obtained from Aldrich Chemical Co. Organometallic solutions were periodically titrated against menthol / phenanthroline.[350] Amino acid derivatives and amino acids were purchased from Sigma Chemical Co.

Where possible , the progress of reactions was monitored by thin-layer chromatography (TLC) using one or more of the following methods of development: UV absorption by fluorescence quenching; iodine staining, ninhydrin spray for amino acids, bromocresol green spray for acids and *p*-dimethylaminobenzaldehyde/HCl/ethanol spray

for hydrazino compounds. For TLC of amino acids on ion exchange resin, sodium citrate buffer was used as a solvent. When relatively non-volatile solvents such as water or dimethylformamide (DMF) were employed in reaction mixtures the TLC plate was heated or dried *in vacuo* before development. Commercial thin layer and preparative thin layer chromatography plates used were: normal silica, Merck 60 F-254; reverse-phase, Merck RP-8 F-254S; ion exchange resin, Polygram®Ionex-25 SA-Na. Silica gel for column chromatography was Merck type 60, 70-230 mesh or its equivalent from General Intermediates of Canada. Flash chromatography was performed according to the method of Still[351], using Merck type 60, 230-420 mesh silica gel and nitrogen pressure. All solvent mixtures listed were volume ratios. For ion exchange chromatography, cation and anion exchange resins were purchased from BioRad. Microcrystalline cellulose for cellulose chromatography was purchased from Merck and was packed and eluted under low pressure (3-5 psi). Compound detection after chromatography on cellulose was performed by TLC following removal of the solvents from a portion of each fraction in a Savant™ speed vac concentrator.

All literature compounds had infrared (IR), ^{1}H NMR, and mass spectra (MS) consistent with the assigned structures. Melting points are uncorrected and were determined on a Thomas Hoover or Büchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on a Perkin Elmer 241 polarimeter using a microcell (10.00 cm path length, 0.9 mL volume) at ambient temperature. All specific rotations reported were measured at the sodium D line. IR spectra were recorded on a Nicolet 7199 FT-IR spectrometer. Mass spectra were recorded on a Kratos AEI MS-50 (high resolution, electron impact ionization), MS-12 (chemical (NH_3) ionization, CI NH_3), and MS-9 (fast atom bombardment with argon, Pos FAB) instruments. The term Cleland, used as a matrix in Pos FAB spectra, refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained using a Perkin Elmer 240 CHN analyzer.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WP-80 (continuous wave), WH-200, AM-300, WM-360, or WH-400 instruments in the specified solvent. Tetramethylsilane (TMS) (or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for aqueous samples) was used as an internal standard for ^{1}H NMR spectra. Deuterium NMR spectra were obtained on the WH-400 spectrometer, recorded at 61.4 MHz, proton decoupled and either unlocked or locked on C_6F_6, trifluoroacetic acid (TFA), or sodium trifluoroacetate. ^{13}C NMR spectra were obtained on the WH-200, AM-300, WM-360, or WH-400 instruments and the deuterated solvent peak was used as the internal reference.

Gas chromatographic (GC) analysis of the camphanamide esters was performed on a Hewlett Packard 5890 Gas Chromatograph equipped with an Alltech fused silica open tubular (FSOT) megabore column (10m x 0.53 mm) RSL 300 1.2mm inside diameter. Column conditions are described later.

For the enzyme work, NADP and bovine serum albumin were obtained from Sigma, and Spectropor dialysis tubing sizes 2 and 4, Scintiverse E scintillation cocktail and Tris were obtained from Fisher. Dithiothreitol (DTT) was purchased from Diagnositic Chemicals Ltd and Schwarz-Mann Biotech (ICN Biomedicals). DEAE cellulose was obtained from Whatman and Matrex Green A resin from Amicon. Q-Sepharose was purchased from Pharmacia. [G-^{3}H] DAP (statistical mixture of isomers 1.09 Ci/ mmol) was purchased from Amersham. Spectrophotometric assays were performed on a Hewlett Packard(HP) 8452A diode array spectrophotometer equipped with a HP Vectra ES computer loaded with kinetics and quantitation programs as part of the HP89530A MS DOS-UV/VIS operating software. Radioactivity was determined on a Beckman LS5000TD scintillation counter using Scintiverse E scintillation cocktail. Electrophoresis was performed using a BioRad 3000 Xi computer-controlled power supply and either a Hoefer vertical slab unit or a BioRad MinProtean vertical slab unit. Acrylamide gel reagents were purchased from BioRad

2,6-Bis-(*N*-carbobenzyloxy)amino-1,7-heptanedioic Acid (19) [86]

The procedure of Wade and coworkers[86] was followed. Benzyl chloroformate (95%, 45.0 mL, 299 mmol) was added dropwise over 40 min to a cold (0 °C) solution of diaminopimelic acid (**1**) (20.0 g 105 mmol) in 2.5 M NaOH (200 mL). The mixture was warmed to room temperature and was stirred for 3.5 h. Additional benzyl chloroformate (6.00 mL, 42.0 mmol) was added and the reaction was stirred a further 0.5 h. The solution was extracted with ethyl acetate (EtOAc) (2 x 100 mL) and the aqueous phase was acidified to pH 1 with HCl. The resultant cloudy solution was extracted with EtOAc (3 x 250 mL) and the combined organic extracts were dried over Na_2SO_4. Evaporation of the EtOAc *in vacuo* and trituration of the residue with hexanes afforded 47.8 g (99%) of a white amorphous solid: IR (CH_3OH cast) 3320, 1720, 1714, 1528, 1231, 1216, 1062, 1028 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.42-7.26 (m, 10H, ArH), 5.09 (overlapping s, 2H, CH_2Ar), 5.08 (overlapping s, 2H, CH_2Ar), 4.18 (dd, 2H, *J*= 9.2, 4.9 Hz, CHNH),1.98-1.42 (m, 6H, 3 CH_2); MS (Pos FAB, glycerol) 459.23 (459.17 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Di-*t*-butyl 2,6-Bis(*N*-carbobenzyloxy)aminoheptanedioate (20) [220]

The procedure of Bricas and coworkers[220] was modified. A solution of **19** (30.0 g, 65.5. mmol) and concd H_2SO_4 (4 mL) in CH_2Cl_2 (300 mL) was placed in a glass sleeve inside a Parr bomb. The bomb was sealed and was cooled to - 22 °C before isobutylene (92.0 mL (- 78 °C), 975 mmol) was distilled into the bomb. The reaction vessel was sealed, and was warmed briefly to 82 °C before cooling to 48-55 °C. This temperature was maintained for 72 h. The bomb was cooled to room temperature and the excess isobutylene was vented through 10% $KHCO_3$ (100 mL). The solution in the bomb was washed with 10% $KHCO_3$ (300 mL) and water (2 x 150 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed *in vacuo* to give 30.6 g (82%) of a golden oil: IR ($CHCl_3$ cast) 3340,

2950, 1723, 1523, 1155 cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) 7.21 (s, 10 H, ArH), 5.18 (br d, 2H, J= 7.0 Hz, 2 NH), 5.00 (s, 4H, 2 CH_2Ar), 4.27-3.90 (m, 2H, 2 CHNH), 2.00-1.03 (m, 6H, 3 CH_2), 1.60 (s, 18H, 2 $C(CH_3)_3$); exact mass 514.2291 (514.2315 calcd for $C_{31}H_{42}N_2O_8$ -C_4H_8), MS (CI NH_3) 588 (588 calcd for $M^+ + NH_4^+$). Purity of the material was determined to be greater than 95% by ^1H NMR.

di-*tert*-Butyl 2-(*N*-Carbobenzyloxy)amino-6-aminoheptanedioate (21)[80]

The procedure of Kelland[80] was modified. Compound **20** (7.59 g 13.3.mmol) in iPrOH (76 mL) was added to moistened 5% Pd on carbon (0.77 g) in a 3 necked flask fitted with a bent tube of crushed KOH. The apparatus was flushed with H_2 and was stirred under H_2 (1 atm) for 1 h. The reaction mixture was filtered through Celite, and the Celite was washed with EtOAc. The combined filtrates were concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc) to give 2.52 g (33%) starting material **62** and 2.32 g (40%) of an oil: IR ($CHCl_3$ cast) 3350, 2968, 1725, 1155 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.45-7.25 (m, 5H, ArH), 5.60-5.45 (m, 1H, NH), 5.10 (s, 2H, CH_2Ar), 4.30-4.18 (m, 1H, CHNH), 3.35-3.27 (m 1H, $CHNH_2$), 2.30-1.10 (m, 8H, $CH_2CH_2CH_2$, NH_2), 1.43 (s, 18H, 2 $C(CH_3)_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 175.05 (CO_2R), 174.99 (CO_2R), 171.4 (CO_2R), 155.8 (CONH), 136.3 (Ar), 128.4 (Ar), 127.9 (Ar), 81.9 ($C(CH_3)_3$), 80.9($C(CH_3)_3$), 66.7(CH_2Ar), 54.6 (CHNH), 54.1 ($CHNH_2$) 34.4 (CH_2), 32.45 (CH_2), 32.37 (CH_2), 27.91 ($C(CH_3)_3$), 27.86 ($C(CH_3)_3$), 21.2 (CH_2), 21.1 (CH_2); exact mass 437.2656 (437.2651 calcd for $C_{23}H_{37}N_2O_6$).Purity of the material was determined to be greater than 95% by ^1H NMR.

Monoperoxyphthalic Acid (23) [352]

The procedure of Kelland[80] was used. Recrystallized phthalic anhydride (2.00 g, 13.5 mmol) was added to a cooled (-10 °C) solution of Na_2CO_3 (1.43 g, 13.5 mmol) and hydrogen peroxide (30% , 1.65 mL, 16.2 mmol) in water (10 mL). The mixture was

stirred for 30 min and then was acidified to pH 1 with 17% sulfuric acid. It was extracted with ether (3 x 5.0 mL). The combined ether layers were washed with 40% NH_4SO_4 (2 x 5.0 mL) and dried over $MgSO_4$ in the refrigerator. Aliquots (1.00 mL) of this solution were added to 20% KI (15 mL), and after 10 min the I_2 produced was titrated against 0.10 M $Na_2S_2O_3$. Concentration of monoperoxyphthalic acid was found to be 0.37 M (82% yield).

Di-*tert*-butyl 2-(*N*-Carbobenzyloxy)amino-6-hydroxylaminoheptanedioate (25) [57]

Anisaldehyde (1.85 mL,15.2 mmol) and Na_2SO_4 (5.00 g 35.2 mmol) were added to a solution of **21** (6.60 g, 15.1 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 23 h. Evaporation of the solvent *in vacuo* yielded 8.67 g of a thick oil. The oil was dissolved in ether (40 mL) and 0.25M **63** in ether (84.0 mL) was added. The mixture was left to stand in the refrigerator for 5.25 h and the solvent was removed *in vacuo* to afford a thick yellow oil. This oil was dissolved in $CHCl_3$ and left to stand in the refrigerator for 1 h. The slurry was filtered and the precipitate was washed with $CHCl_3$. The filtrate was concentrated *in vacuo* to give 9.30 g of a viscous pale yellow oil which was dissolved in 95% EtOH (50 mL). Hydroxylamine hydrochloride (1.45 g 20.9 mmol) was added and the mixture was heated gently for 10 min to dissolve most of the salt. The solution was swirled for 15 min and then was left at room temperature for 30 min. Evaporation of the solvent *in vacuo* gave a residue which was dissolved in $CHCl_3$ (1 mL). This was evaporated *in vacuo*, re-dissolved in ether, and was then concentrated *in vacuo* to yield an oily yellow solid. Flash chromatography (35% Et_2O / CH_2Cl_2 followed by 60% EtOAc / 40% hexanes) afforded 1.96 g (29%) of a colorless oil: IR ($CHCl_3$ cast) 3540, 2975, 1727, 1155 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5H, ArH), 5.57-5.49 (m, 1H NH), 5.08 (s, 2H, CH_2Ar), 4.29-4.16 (m, 1H, CHNH), 3.50-3.37 (m, 1H, CHNHOH), 1.90-1.20 (m, 8H, $CH_2CH_2CH_2$, NHOH), 1.47 (s, 9H, $C(CH_3)_3$), 1.45

(s, 9H, $C(CH_3)_3$); ^{13}C NMR (360MHz, $CDCl_3$) δ 172.9 (CO_2R), 172.8 (CO_2R), 171.52 (CO_2R), 171.48 (CO_2R), 155.9 (CONH), 136.22 (Ar), 128.4 (Ar), 128.0 (Ar), 82.0 ($C(CH_3)_3$), 81.6 ($C(CH_3)_3$), 66.8 (CH_2Ar), 65.34 (CHNH), 65.26 (CHNH), 54.0 (CHNHOH), 53.9 (CHNHOH), 32.5 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 28.0 ($C(CH_3)_3$), 27.9 ($C(CH_3)_3$), 21.4 (CH_2), 21.3 (CH_2); exact mass 452.2531 (452.2522 calcd for $C_{23}H_{36}N_2O_7$). Purity of the material was determined to be greater than 95% by ^1H NMR.

2-Amino-6-hydroxylaminoheptanedioic Acid (3) [57]

The procedure of Kelland *et al.*[57] was followed. Compound **25** (1.63 g, 3.60 mmol) was dissolved in cold (0 °C) AcOH (4 mL) and a saturated HBr / AcOH solution (4 mL) was added. The solution was stirred for 15 min at 0 °C, and was then warmed to room temperature and stirred for 25 min. Water (17 mL) was added and the solution was concentrated *in vacuo* to a brown oil. The oil was dissolved in water and was loaded onto an ion exchange column (AG50W X8, H^+form, 40 mL). The column was washed with water (50 mL) and eluted with 0.5% NH_4OH (50 mL), 1.0% NH_4OH (50 mL) and 2% NH_4OH (about 100 mL). Those fractions containing amino acid were combined and concentrated *in vacuo*. Subsequent lyophilization produced 0.726 g (98%) of an amorphous solid:IR (KBr disk) 3410, 3210, 3030, 2940, 1598, 1403 cm^{-1}; ^1H NMR (400 MHz, D_20) δ 3.80-3.66 (m, 1H, CHNHOH), 3.58-3.46 (m, 1H, $CHNH_2$), 2.00-1.77 (m, 2H, CH_2CHNOH), 1.77-1.54 (m, 2H, CH_2CHNH_2), 1.54-1.30 (m, 2H, CH_2); ^{13}C NMR (90.5 MHz, D_2O) δ 179.3 (CO_2R), 175.53 (CO_2R), 175.51 (CO_2R), 67.3 (CHNHOH), 67.2 (CHNHOH), 55.4 ($CHNH_2$), 31.25 (CH_2), 31.18 (CH_2), 29.00 (CH_2), 28.96 (CH_2), 22.0 (CH_2), 21.8 (CH_2); MS (FAB) 207.23 (207.09 calcd for $C_7H_{15}N_2O_5$). Purity of the material was determined to be greater than 95% by ^1H NMR.

4,5-Diphenyl-1,3-dioxol-2-one (27)

The procedure of Sheehan and Guziec[224] was used. Phosgene (38.0 mL (-78 °C), 530 mmol) was added by distillation through a cannula to a cooled (4 °C), stirred suspension of benzoin (106 g, 500 mmol) in benzene (400 mL). Once the addition was complete, *N, N*-dimethylaniline (64.0 mL, 500. mmol) was added dropwise over 1 h. The mixture was warmed to room temperature and stirred for 19 h. The mixture was cooled in ice and was filtered to remove the *N, N*-dimethylaniline hydrochloride salt. The salt was washed with benzene (2 x 40 mL) and the combined benzene fractions were heated to reflux for 3 h. The solution was washed with 0.5 M HCl (250 mL), water (250 mL), and brine (100 mL), and was then dried over Na_2SO_4 . Evaporation *in vacuo* produced 121 g of a blue-green oil which was dissolved in 20 mL hot 95% EtOH and left at room temperature overnight to give 63.7 g crystals. Recrystallization from 95% EtOH yielded 61.2 g (34%) colorless needles: mp 74-75 °C (lit.[224] mp 75-76 °C); IR ($CHCl_3$ cast) 1815, 1237, 1208, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.25 (m, 10H, ArH); exact mass 238.0630 (238.0630 calcd for $C_{15}H_{10}O_3$). Anal. Calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.74; H, 4.39.

2-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioic Acid (28) [57]

The procedure of Kelland *et al.*[57] was used. Tetramethylammonium hydroxide (20% in methanol, 105 mL, 230 mmol.) was added to D,L-α-aminopimelic acid (**26**) (20.0 g, 114 mmol.). The mixture was warmed slightly on a steam bath, and was then concentrated to dryness *in vacuo*. The residue was suspended in DMF (118 mL) and **27** (27.2 g, 114 mmol) was added. The mixture was stirred at room temperature for 1.5 h, and then acidified with 2 M HCl (115 mL). The mixture was extracted wtih EtOAc (500 mL), the EtOAc fraction was washed with water (3 x 350 mL). The organic extract was dried over Na_2SO_4 and concentrated *in vacuo* to a yellow amorphous solid. This residue was dissolved in trifluoroacetic acid (115 mL) and was stirred for 2.5 h. The trifluoroacetic

acid was removed *in vacuo* and the residue was partitioned between CH_2Cl_2 (375 mL), water (3 x 150 mL) and brine (150 mL). The organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. Crystallization of the resultant residue from EtOAc / hexanes yielded 31.4 g (70%) white granular crystals: mp 131.5-133.5 °C (lit.[57] mp 132-132.5 °C); IR ($CHCl_3$ cast) 3060, 2940, 1759 1718 cm^{-1}; ^1H NMR (360 MHz, CD_3OD), δ 7.70-6.98 (m, 10 H, ArH), 4.06 (dd, 1H, *J*=11.0, 5.0 Hz, CHN), 2.05 (t, 2H, *J*=3.6 Hz, CH_2CO_2H), 2.00-1.85 (m, 1H, CH_aHCHN),1.85-1.70 (m, 1H, CHH_bCHN), 1.36-0.93 (m, 4H, CH_2CH_2); ^{13}C NMR (90.5 MHz, CD_3OD) 177.1 (CO_2H), 172.2 (CO_2H), 155.6 (CON),136.1 (Ar), 132.2 (Ar), 131.7 (Ar), 130.8 (Ar), 129.5 (Ar), 129.0 (Ar), 128.8 (C=C), 127.9 (C=C), 125.4 (Ar), 125.3(Ar), 57.1 (CHCN), 34.5 (CH_2CO_2H), 28.9 (CH_2), 25.8 (CH_2), 25.2 (CH_2); exact mass 395.1374 (395.1369 calcd for $C_{22}H_{21}NO_6$). Anal. Calcd for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.65; H, 5.36; N, 3.51.

(2*R*)-2-Amino-3-methyl-1-butanol (30)

The procedure of Hsuno *et al.*[226] was modified. A mixture of lithium aluminum hydride (12.9 g, 342 mmol) and dry THF (1.00 L) was heated to reflux under an argon atmosphere for 30 min, and was then stirred and cooled in ice while D-valine (**29**) (22.9 g, 196 mmol) was added in small portions. The mixture was heated to reflux for 17 h and was quenched by the sequential addition of Celite (20.2 g), water (20 mL), 10% NaOH (13 mL), and water (60 mL). The slurry was filtered, and the precipitate was washed with EtOAc (2 x 100 mL). The combined filtrates were concentrated *in vacuo* to yield a clear yellow oil. Distillation of the oil under reduced pressure afforded 12.9 g (64 %) of a clear colorless oil: bp 68-71 °C at 4 mm Hg (lit.[226] bp 55-57 °C at 2 mm Hg); $[\alpha]_D$= -15.2 ° (c=5.0, $CHCl_3$) (lit.[353] $[\alpha]_D$= +14.6 ° (neat) for S isomer); IR ($CHCl_3$ cast) 3342, 3298, 3249, 2956, 2871, 1622, 1404, 1387, 1068 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 3.64 (dd, 1H, *J*= 10.4, 3.7 Hz, CH_aHOH), 3.30 (dd, 1H, *J*= 10.4, 8.6 Hz, CHH_bOH), 2.65-

2.55 (m, 1H, $CHNH_2$), 2.09 (br s, 2H, NH_2), 1.65-1.52 (m, 1H, $CH(CH_3)_2$), 0.92 (2 overlapping d, 6H, J= 4.9 , 4.3 Hz, $CH(CH_3)_2$) exact mass 104.1073 (104.1071 calcd for $C_5H_{13}NO$ +H). Purity of the material was determined to be greater than 95% by ^1H NMR.

(4*R*)-4-Isopropyl-1,3-oxazolidin-2-one (31)

The procedure of Evans *et al.*[158] was used. A mixture of **30** (19.3 g, 187 mmol), diethyl carbonate (25.0 mL, 206 mmol), and K_2CO_3 (2.66 g, 19.2 mmol) were heated to reflux for 1.75 h with the concurrent removal of EtOH from the reaction mixture. The residue was dissolved in ether (500 mL) and filtered through Celite. The filtrate was concentrated *in vacuo* and left to crystallize. This yielded 20.9 g (87%) of colorless needles: mp 70.5-71.5 °C (lit.[225] mp 71-72 °C); $[\alpha]_D$= -16.7 °, (c=9.0, $CHCl_3$) (lit.[225] $[\alpha]_D$= +14.8 ° for S isomer (c=7.0 $CHCl_3$)); IR ($CHCl_3$ cast) 3240, 1747, 1247 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 6.24 (br s, 1H, NH), 4.45 (dd, 1H, J= 8.6, 8.6 Hz, CHO), 4.11 (dd, 1H, J= 8.5, 6.1 Hz, CHHO), 3.62 (m, 1H, CHNH), 1.74 (m, 1H, $CH(CH_3)_2$), 0.97 (d, 3H, J= 6.7 Hz, $CHCH_3$), 0.91 (d, 3H, J= 7.3 Hz, $CHCH_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 160.6 (C=O), 68.4 (CO), 58.2 (CN), 32.5 ($C(CH_3)_2$), 17.7 (CH_3), 17.4 (CH_3); exact mass 129.0790 (129.0790 calcd for $C_6H_{11}NO_2$) Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84 Found: C, 55.58; H,8.62; N, 10.66.

3,3'-[(2*S*)-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(4*R*)-4-isopropyl-1,3-oxazolidin-2-one] (33),
3,3'-[(2*R*)-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(4*R*)-4-isopropyl-1,3-oxazolidin-2-one] (32),
and 3,-[(2*RS*)-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-[(4*R*)-4-isopropyl-1,3-oxazolidin-2-one] (34)

The method of Kelland[80] was followed. Freshly distilled oxalyl chloride (4.50 mL, 52.4 mmol) followed by dry CH_2Cl_2 (25 mL)was added to a cooled (0 °C) suspension of **28** in dry benzene (25 mL). The mixture was stirred at room temperature for 1.75 h. The solvents were removed *in vacuo* and the residue was triturated with benzene (2 x 10 mL). This oil was then dissolved in dry THF (10 mL) under Argon. n-Butyl lithium (1.47 M in hexanes) was added to a cooled (0 °C) solution of **31** (3.33 g, 25.8 mmol) and 1,10 phenanthroline (2 crystals) in dry THF (40 mL) until an orange sludge was obtained. The solution of the acid chloride was added and the solution was stirred for 10 min before the reaction was quenched with 10% NH_4Cl (10 mL). The THF layer was removed and the aqueous layer was extracted with CH_2Cl_2 (2 x 80 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. Flash chromatography (40% EtOAc / 60% hexanes, then 50% EtOAc / 50% hexanes, followed by 1% AcOH / 99% EtOAc) separated the two diastereomers **32** and **33** from unreacted **31** and 2.17 g (33%) 1/2 acid-1/2 oxazolidinone **34**. Repeated flash chromatography (40% EtOAc / 60% hexanes) afforded 2.47 g (31%) of **32** and 2.24 g (28%) of **33** as amorphous solids. For **32**: $[\alpha]_D$= - 11.6 ° (c=3.1, CH_2Cl_2) (lit.[80] $[\alpha]_D$= - 11.4 ° (c= 4.5, CH_2Cl_2); IR ($CHCl_3$ cast) 2960, 2930, 2870,1777, 1706, 1600, 1388, 1364, 1302, 1261, 1207 cm^{-1}; ^1H NMR ($CDCl_3$ 360 MHz) 7.58-7.39 (m, 5H, ArH), 7.27-7.13 (m, 5H, ArH), 5.24 (dd, 1H, *J*=10.4 , 4.3 Hz, CH), 4.48-4.37 (m, 2H, 2 CH_aHO), 4.32-4.22 (m, 2H, 2 CH_bHO), 4.22-4.15 (m, 2H, CHN), 3.00-2.80 (m, 2H, CH_2CON), 2.45-2.27 (m, 3H, CH_aHCHN, 2 $CH(CH_3)_2$), 1.97-1.85 (m, 1H, CHH_bCHN), 1.73-1.42

(m, 4H, CH_2CH_2), 0.89 (d, 6H, J=7.0 Hz, $CH(CH_3)_2$), 0.85 (d, 6H, J=7.0 Hz, $CH(CH_3)_2$); ^{13}C NMR (90.5 MHz, $CDCl_3$) 172.7 (CO), 169.3 (CO), 154.0 (CO), 153.0 (CO), 134.8 (Ar), 130.9 (Ar), 130.3 (Ar), 129.5 (Ar), 128.3 (Ar), 127.6 (C=C), 124.4 (Ar), 123.5 (C=C), 64.1 (CH_2O), 63.3 (CH_2O), 58.5 (CHN), 58.3 (CHN), 57.4 (CHCN), 34.9 (CH_2CO), 28.5 ($CH(CH_3)_2$), 28.4 ($CH(CH_3)_2$), 28.2 (CH_2), 25.9 (CH_2), 23.4 (CH_2), 17.9 (CH_3), 17.7 (CH_3), 14.8 (CH_3), 14.6 (CH_3); exact mass 617.2743 (617.2737 calcd for $C_{34}H_{39}N_3O_8$).Anal. Calcd.for $C_{34}H_{39}N_3O_8$: C, 66.11; H, 6.36; N, 6.80. Found: C, 66.20; H, 6.56, N; 6.58.

For **33**: $[\alpha]_D$= - 109.7 ° (c=5.0, CH_2Cl_2) (lit.[80] $[\alpha]_D$= - 100.2 ° (c=4.0, CH_2Cl_2); IR ($CHCl_3$ cast) 2960, 2930, 2870,1777, 1703, 1388, 1206 cm^{-1};^1H NMR ($CDCl_3$ 360 MHz) 7.60-7.36 (m, 5H, ArH), 7.31-7.16 (m, 5H, ArH), 5.19 (dd, 1H, J= 10.7, 4.0 Hz, CH), 4.46-4.36 (m, 2H, 2 CH_aHO), 4.33-4.15 (m, 4H, 2 CHH_bO, 2 CHN), 3.00-2.84 (m, 2H, CH_2CO), 2.66-2.28 (m, 2H, 2 $CH(CH_3)_2$), 1.89-1.76 (m, 1H, CH_aHCHN), 1.73-1.39 (m, 5H, CH_2CH_2, CHH_bCHN), 0.98-0.70 (m, 12H, CH_3); ^{13}C NMR (90.5 MHz, $CDCl_3$) 172.7 (CO), 169.2 (CO), 154.1 (CO), 154.0 (CO), 153.0 (CO), 134.6 (Ar),130.3 (Ar), 129.6 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (C=C), 124.3 (Ar), 123.6 (C=C), 63.6 (CH_2O), 63.3 (CH_2O), 59.1 (CHN), 58.3 (CHN), 57.3 (CHCN), 34.8 (CH_2CO), 28.3 ($CH(CH_3)_2$), 27.8 ($CH(CH_3)_2$), 27.6 (CH_2), 26.0 (CH_2), 23.3 (CH_2), 17.9 (CH_3), 14.6 (CH_3),14.2 (CH_3); exact mass 617.2725 (617.2737 calcd for $C_{34}H_{39}N_3O_8$). Anal. Calcd.for $C_{34}H_{39}N_3O_8$: C, 66.11; H, 6.36; N, 6.80. Found: C, 66.39; H, 6.35, N; 6.76.

For **34**: IR ($CHCl_3$ cast) 3060,2964, 2870, 1762, 1703, 1670, 1386, 1207 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.64-7.35 (m, 5H, ArH), 7.30-7.13 (m, 5H, ArH), 5.10-4.46 (br s, CO_2H), 4.46-4.34 (m, 1H, CHNR), 4.34-4.07 (m, 3H, CH_2O, CHN), 3.20-2.71 (m, 2H, CH_2CO)2.43-1.86 (m, 3H, CH_2CHN, $CHCH_3$), 1.71-1.13 m, 6H, $CH_2CH_2CH_2$), 1.00-0.84 (m, 6H, CH_3); exact mass 506.2058 (506.2053 calcd for $C_{28}H_{30}N_2O_7$). Purity of the material was determined to be greater than 95% by ^1H NMR.

3,3'-[(2R, 6S)-2-(1,2-Bis(benzyloxycarbonyl)hydrazino)-6-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(4R)-4-isopropyl-1,3-oxazolidin-2-one] (35) [57]

The procedure of Kelland[80] was followed. n-BuLi (1.38M) in hexanes (3.00 mL, 4.14 mmol) was added to a cooled (-78 °C) solution of diisopropylamine (0.580 mL, 4.14 mmol) in dry THF (30 mL) and the mixture was stirred for 0.5 h. A solution of **33** (2.54 g, 4.11 mmol) in dry THF (15 mL) was added, followed by dibenzyl azodicarboxylate (1.70 g, 5.70 mmol) in dry THF (10 mL). The mixture was then quenched with 10% NH_4Cl (20 mL) and the organic layer removed. The aqueous phase was extracted with CH_2Cl_2 (4 x 100 mL) and the combined organic phases were dried over Na_2SO_4. Evaporation of the solvent *in vacuo* gave an amorphous solid which was purified by flash chromatography (50% EtOAc / 50% hexanes) to give 1.11 g (29%) of an amorphous solid:$[\alpha]_D$= -13.5 ° (c=26, CH_2Cl_2) (lit.[80] $[\alpha]_D$= -14.0 ° (c=7.7, CH_2Cl_2)); IR ($CHCl_3$ cast) 3320, 1780, 1763, 1714, 1389, 1304, 1247, 1213 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 7.85-6.90 (m, 20H, ArH), 5.80 (br s, 1H,CHNR), 5.40-5.00 (m, 5H, CHNRNHR, 2 CH_2Ar), 4.36-4.09 (m, 6H, 2 CHN, 2 CH_2O), 2.40-2.12 (m, 2H, 2 $CH(CH_3)_2$) 2.12-1.50 (m, 6H, 3 CH_2), 0.87 (d, 6H,*J*=7.3 Hz, $2CHCH_3$), 0.83 (d,*J*=7.3 Hz, 6H, $2CHCH_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) 169.5 (CO), 154.1 (CO), 153.2 (CO), 153.0 (CO), 136.0 (Ar), 134.8 (Ar), 131.1 (Ar), 130.1 (Ar), 129.4 (Ar), 128.4 (Ar), 128.3 (Ar), 127.5 (Ar), 127.2 (C=C), 124.5 (Ar), 123.6 (C=C), 68.3, (CHN), 67.5 (CHN), 64.1 (CH_2O), 63.8 (CH_2O, 61.0 (CH_2Ar), 60.1 (CH_2Ar), 59.5 (CHN), 57.0 (CHN), 28.4 ($CH(CH_3)_2$), 28.0 ($CH(CH_3)_2$), 27.9 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 17.8 (CH_3), 17.7 (CH_3), 15.0 (CH_3), 14.3 (CH_3); MS (Pos FAB, Cleland) 916.38 (916.38 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dibenzyl 2-[1,2-Bis(benzyloxycarbonyl)hydrazino]-6-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioate (36) [57]

The method of Kelland[80] was followed. A cooled (0 °C) solution of benzyl alcohol (0.066 mL, 0.638 mmol) and 1.38M n-BuLi (0.425 mL, 0.587 mmol) in dry THF was added to a cooled (0 °C) solution of **35** (0.269 g, 0.293 mmol) in dry THF (3 mL). The mixture was stirred for 55 min before it was quenched with 10% NH_4Cl (1.5 mL). The solution was extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were dried over Na_2SO_4. Concentration *in vacuo* afforded a pale yellow oil which upon flash chromatography (30% EtOAc / 70% hexanes) yielded 0.134 g (52%) of a colorless oil: $[\alpha]_D$= -5.3 ° (c=13, CH_2Cl_2); IR (CH_2Cl_2 cast) 3300, 1761, 1745, 1716, 1293,1271, 1215 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.62-7.08 (m, 30H, ArH),6.84-6.70 (br d, 1H, NH), 5.00-4.84 (m, 9H, CH_2Ar, CHNNH), 4.20-4.00 (m, 1H, CHN), 2.25-1.00 (m, 6H, 3 CH_2); MS (Pos FAB, Cleland), 874.27 (874.30 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

2-Amino-6-hydrazinoheptanedioic Acid Hydrochloride (4) [57]

The procedure of Kelland[80] was used. A solution of **36** (0.106 g, 0.121 mmol) in MeOH (10 mL), and EtOAc (7 drops) was added to a suspension of 5% Pd on carbon (0.0625 g) in 3M HCl (1mL) in a Parr bottle. The reaction vessel was flushed with H_2 before it was shaken for 24 h at room temperature and 48 psi. The mixture was filtered through Celite, the Celite was washed with MeOH and the filtrate was evaporated *in vacuo* to give an amorphous solid. This was placed on an ion exchange column (AG50Wx8, H^+ form , 2.5 mL) and eluted with water (20 mL) and 1M HCl (300 mL) to give 0.0350 g (quant.) of a hygroscopic amorphous solid: IR (neat film) 2945, 1792, 1591, 1494 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.00-3.91 (m, 1H, $CHNHNH_2$), 3.77-3.66 (m, 1H, $CHNH_2$), 1.94-1.56 (m, 4H, $CH_2CH_2CH_2$), 1.56-1.30 (m, 2H, CH_2); MS (FAB) 278.18 (278.06 calcd for MH^+ + 2HCl). Purity of the material was determined to be greater than 92% by ^1H NMR.

Alternative Route to 2-Amino-6-hydrazinoheptanedioic Acid (4)

Compound **128** (35.3 mg, 0.0611 mmol) was suspended in 5.7 M HCl (5 mL) and heated to reflux for 2 h. The acid was removed in vacuo and the residue purified by cellulose chromatography (4:2:1 n-butanol: H_2O: AcOH) to give 10.2 mg (81%) of a foam: IR (neat film) 3400, 3140, 1658, 1400 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.18 (dd, 1H, *J*= 7.1, 4.8 Hz $CHNHNH_3^+$), 3.94-3.84 (m, 1H, $CHNH_3^+$), 2.02-1.68 (m, 4H, $CH_2CH_2CH_2$), 1.54-1.30 (m, 2H, CH_2); MS (Pos FAB, glycerol HCO_2H) 206.44 (206. 11 calcd for $C_7H_{15}N_3O_4$ +H^+).

Methyl γ-Glutamic Acid (38)

The method of Hanby *et al.*[248] was followed. L-Glutamic acid (**37**) (36.8 g, 250 mmol) was added to a stirred, cooled (0 °C) solution of acetyl chloride (20.0 mL, 281 mmol) in MeOH (250 mL) The solution was stirred at room temperature for 21 h. Pyridine (25.0 mL, 309 mmol) was added and the resultant slurry was stirred for 16.5 h and then was left to stand, stoppered, at room temperature for 6 days. The mixture was filtered, and the colorless crystals were washed once with EtOH, and twice with ether to give 12.2 g (25%) white crystals of the hydrochloride salt. Concentration and crystallization of the filtrate afforded a further 8.02 g (16%) of the hydrochloride salt: mp 170-171 °C (lit.[354] mp 167 °C); $[\alpha]_D$= +24.4 ° (c= 1.1, H_2O); IR (KBr disk) 3140, 2940,1733, 1439,1256, 1201, 1079 cm^{-1} ;^1H NMR (400 MHz, D_2O) δ 3.78 (t, *J*=6 Hz, 1H, $CHNH_2$), 3.71 (s, 3H, OCH_3), 2.67-2.50 (m, 2H, CH_2), 2.25-2.08 (m, 2H, CH_2); exact mass 162.0765 (162.0766 calcd for $C_6H_{12}NO_4$). Purity of the material was determined to be greater than 95% by ^1H NMR.

γ-Methyl (*N*-Carbobenzyloxy)glutamate (39)

The procedure of Hanby *et al*[248] was used. Benzyl chloroformate (95%, 7.50 mL, 49.9 mmol) was added dropwise over 15 min to a cooled (0 °C) solution of **38** (5.00 g 25.3 mmol) and $NaHCO_3$ (5.66 g, 67.4 mmol) in water (65 mL). During the addition of the benzyl chloroformate the pH of the solution was raised from pH 7 to pH 10 with the addition of 10 M NaOH. The mixture was stirred for 1.25 h, during which the pH was readjusted with 10 M NaOH, and the solution was stirred an additional 3.75 h at room temperature. The mixture was extracted with ether (2 x 30 mL), and the aqueous phase was acidified to pH 1 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x mL) and the combined EtOAc extracts were dried over Na_2SO_4. Concentration *in vacuo* yielded a colorless oil. A portion (4.00 g) was purified by flash chromatography (65% EtOAc / 35% hexanes) to give 3.08 g (77%) of a colorless solid. Recrystallization from CCl_4 afforded 2.49 g (81%) of a colorless powder: mp 66-66.5 °C (lit.[248] mp 72-73 °C); $[\alpha]_D$= -15.2 ° (c=7.7, 1.4N $KHCO_3$) (lit.[248] $[\alpha]_D$= -15.3 ° (c= 7.5, 1.4 N $KHCO_3$)); IR($CHCl_3$ cast) 3330, 1734, 1718, 1540, 1214 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 5H, ArH), 5.55 (d, 1H, *J*=8.0 Hz, NH), 5.12 (s, 2H, CH_2Ar), 4.44 (dd, 1H, *J*=13.0, 7.9 Hz, CHNH), 3.66 (s, 3H, CH_3), 2.58-1.97 (m, 4H, CH_2CH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ 175.8 (CO_2R), 173.5 (CO_2R), 156.2 (CONH), 135.9 (Ar), 128.5 (Ar), 128.2 (Ar), 128.0 (Ar), 67.2 (CH_2Ar), 53.1 (CHNH), 51.8 (OCH_3), 30.0 (CH_2), 27.2 (CH_2); exact mass (295.1056 calcd for $C_{14}H_{17}NO_6$). Anal. Calcd for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.54; H, 5.73; N,4.84.

(2*S*) *N*-Carbobenzyloxy-γ-glutamyl Hydrazide (40)

The procedure of Greenstein and Winitz[246] was modified. A mixture of **39** (5.75 g, 19.5 mmol) and hydrazine hydrate (5.7 mL, 117 mmol) was heated on a steam bath for 15 min and then was added to ice cold water (100 mL). The pH of the aqueous mixture was acidified to pH 1 using 5.7M HCl was was then adjusted to pH 3.8 using 10M NaOH.

Immediate precipitation of a colorless solid occurred and, after cooling, filtration yielded 4.65 g (81%) of a fine powder. A 0.80 g portion was recrystallized from water to give 0.68 g (85%) fine white needles: mp 177-178 °C (lit[246] mp 177-178 °C); $[\alpha]_D$= -6.8 ° (c= 2.7 , 1.4M $KHCO_3$) ; IR (KBr disk) 3332, 3278, 1722, 1686, 1652, 1536 cm^{-1}; ^1H NMR (300 MHz, D_2O + K_2CO_3) δ 7.48-7.36 (m, 5H, ArH), 5.15 (d, 1H, *J*= 12.4 Hz, CH_aHAr), 5.07 (d, 1H, *J*= 12.4 Hz, CHH_bAr), 3.92 (dd, 1H, *J*= 8.9, 4.5 Hz, CHNH), 2.36-1.78 (m, 4H, CH_2CH_2); MS (CI-NH_3) 296.0 (296.1 calcd for M^++H^+). Anal. Calcd for $C_{13}H_{17}N_3O_5$: C, 52.88; H, 5.80; N, 14.23. Found: C, 52.72; H, 5.64; N, 14.14.

(2*S*) γ Glutamyl Hydrazide (6)

The procedure of Rueppel *et al.*[247] was adapted. Compound **40** (3.85 g, 13.0 mmol) was added to a suspension of 5% Pd on carbon (0.79 g) in 1% AcOH / MeOH (500 mL). The flask was flushed with H_2 and then stirred under H_2 (1 atm) for 2.25 h. The mixture was filtered through Celite and the solvent was removed *in vacuo*. The residue was recrystallized from water / EtOH to afford 1.62 g (77%) of an off-white solid: mp 150-151 °C (lit.[229] mp 165 °C); IR (KBr disk) 3400, 3250, 3040, 1626, 1502, 1311 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 3.76 (dd, 1H, *J*= 12.2, 6.1 Hz, $CHNH_2$), 2.47-2.25 (m, 2H, CH_2CONH), 2.25-2.03 (m, 2H, CH_2CHNH); ^{13}C NMR (75.5 MHz D_2O) δ 174.7 (CO_2H), 174.4 ($CONHNH_2$), 54.9 ($CHNH_2$), 30.4 (CH_2), 27.0 (CH_2); MS (Pos FAB, glycerol) 162.01 (162.08 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dibenzyl Carbonate (41)

The procedure of Overberger *et al.*[355] was modified. Benzyl chloroformate (95%, 50.0 mL, 333 mmol) was added dropwise to a stirred, cooled (0 °C) solution of triethylamine (104 mL, 746 mmol) and benzyl alcohol (40.0 mL, 387 mmol) in toluene (100 mL) over

45 min. The mixture was stirred at room temperature for 1 h, then was filtered and the filtrate washed with water (2.4 L total). The organic phase was dried over $MgSO_4$ and was concentrated *in vacuo*. The remaining liquid was distilled under vacuum (1mm Hg) to give 31.4 g (37%) of a colorless oil: bp 162-164 °C at 1mm Hg (lit.[355] bp 157 °C at 1.1 mm Hg);IR ($CHCl_3$ cast) 1749, 1266, 696 cm^{-1};^1H NMR (80 MHz, $CDCl_3$), δ 7.40 (s, 5H, ArH), 5.20 (s, 2H, CH_2); exact mass 242.0935 (242. 0943 calcd for $C_{15}H_{14}O_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

1-Carbobenzyloxyhydrazine (42)

The procedure of Overberger *et al.*[355] was used. Hydrazine monohydrate (99%, 2.55 mL, 52.5 mmol) was added dropwise with vigorous stirring to a cooled (0 °C) solution of **41** (6.09 g, 25.1 mmol) in 98% EtOH (15mL). The solution was heated to reflux for 45 min, cooled, and the solvent was removed *in vacuo*. The residue was dissolved in ether and cooled to give a white solid. The solid was dissolved in $CHCl_3$ (50 mL) and washed with water (20 mL). Evaporation of $CHCl_3$ yielded 1.49 g white needles, which were recrystallized from benzene / ether to give 0.59 g (14%) white needles: mp 67-68 °C (lit.[355]mp 68-69 °C); IR ($CHCl_3$ cast) 3334, 3303, 1689 cm^{-1}; ^1H NMR δ 7.42 (s, 5H, ArH), 6.10 (br s, 1H, NH), 5.20 (s, 2H, CH_2Ar), 3.82 (br s, 2H, NH_2); exact mass 166.0740 (166.0742 calcd for $C_8H_{10}N_2O_2$). Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07 N, 16.86. Found: C, 57.57; H, 6.00; N, 16.65.

1-Carbobenzyloxy-2-methylhydrazine (43)

The procedure of Pedersen[356] was followed. A solution of **41** (15.0 g, 61.9 mmol) and methylhydrazine (98%, 3.35 mL, 63.6 mmol) in EtOH (15 mL) was heated to reflux for 24 h. The solution was cooled and the solvent was removed *in vacuo*. The remaining benzyl alcohol was removed by vacuum distillation at 1 mm Hg. Refrigeration produced a yellow semi-solid which was washed with hexanes (3 x 25 mL) and then was recrystallized from

cyclohexane to give 2.10 g (19%) of colorless crystals: mp 81-82 °C (lit.[356] mp 81-82 °C); IR (CH_2Cl_2 cast) 3277, 3255, 3237, 2958, 1703, 1569, 1499, 1279, 1149 cm^{-1} ; ^1H NMR (80 MHz, $CDCl_3$) δ 7.40 (s,5H, ArH), 5.22 (s, 2H, OCH_2), 2.74 (s, 3H, CH_3),exact mass 180.0900 (180.0899 calc for $C_9H_{12}N_2O_2$). Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N,15.55. Found: C, 60.02; H,6.85 ; N, 15.73.

Alternative Procedure for 1-Carbobenzyloxy-2-methylhydrazine (43)

The general procedure of Borch and Hassid[249] was adapted. Formaldehyde solution (37%, 0.080 mL. 0.986 mmol) and $NaCNBH_3$ (0.0766g, 1.22 mmol) were added to a solution of **39** in CH_3CN (5 mL) and the mixture was stirred for 4.5 h. During the reaction the pH was periodically adjusted to pH 5 with AcOH. The solvent was removed *in vacuo* and the residue dissolved in 2M NaOH (10 mL). The aqueous solution was extracted with Et_2O (1 x 20 mL, 2 x 10 mL), and the combined organic phases washed with 0.5 M NaOH (1 x 10 mL). The ether was then extracted with 1M HCl (3 x 8 mL). The combined aqueous extracts were neutralized to pH 7 with solid KOH and were extracted with ether (1 x 20mL, 2 x 10 mL). The ether was removed *in vacuo* to give 0.110g (61%) of a cloudy oil. Spectral data was the same as for **43** obtained from reaction of **41** with methylhydrazine.

(2*S*) 1-*tert*-Butyl 5-Methyl (*N*-carbobenzyloxy)glutamate 44 [247]

The method of Bricas *et al.*[220] was adapted. Cold (-78 °C) condensed isobutylene (10.0 mL, 106 mmol) was added to a cooled (-78 °C) stirred solution of **39** (2.18 g, 7.38 mmol) and H_2SO_4 (4 drops) in dry CH_2Cl_2 (30.0 mL) in a pressure bottle. The bottle was sealed and stirred at room temperature for 6 days. The bottle was cooled to -20 °C, opened, and stirred at room temperature for 1.5 h. K_2CO_3 (10%, 10.0 mL) and brine (20.0 mL) were added, and the organic layer was removed. The aqueous layer was extracted with ether (3 x 30.0 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated

in vacuo to afford 2.27 g (88%) of a colorless oil. Purification of a portion (0.505 g, 1.44 mmol) by flash chromatography (30% EtOAc / 70% hexanes), yielded 0.393 g (78%) of a colorless oil: $[\alpha]_D$= +3.1° (c= 30, CH_2Cl_2); IR (CH_2Cl_2 cast) 3350,1737, 1222, 1155 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.42-7.32 (m, 5H, ArH), 5.39 (br d, 1H, *J*=8.0 Hz, NH), 5.12 (s, 2H, CH_2Ar), 4.30-4.15 (m, 1H, CHNH), 3.68 (s, 3H, OCH_3), 2.55-1.84 (m, 4H, CH_2CH_2), 1.45 (s, 9H, $C(CH_3)_3$); exact mass 351.1673 (351.1682 calcd for $C_{18}H_{25}NO_6$). Purity of the material was determined to be greater than 90% by ^1H NMR.

Sodium 1-*tert*-Butyl (2*S*) -*N*-Carbobenzyloxyglutamate (45)

The procedure of Rueppel *et al.*[247] was followed. NaOH in 90% EtOH (0.875M, 3.30 mL, 2.89 mmol) was added to **44** (1.06g, 3.02 mmol) followed by 80% EtOH (7.00 mL), and the mixture was stirred at room temperature for 19 h. Evaporation of the solvent *in vacuo* yielded a cloudy oil. Successive addition and evaporation of EtOH (4 x 15.0 mL), benzene (3 x 15.0 mL), and cyclohexane *in vacuo* afforded 0.88 g (81%) of a white solid: mp 70-72 °C (lit.[247] mp 63-67 °C); $[\alpha]_D$= -9.2 ° (c= 0.44, benzene); IR (KBr disk) 3420, 1720, 1578, 1408, 1155 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 7.45 (s, 5H, ArH), 5.14 (br d, *J*= 4.0 Hz, NH), 4.06-3.94 (m, 1H, $CHNH_2$), 2.33-2.17 (m, 2H CH_2CO_2Na), 2.15-1.80 (m, 2H, CH_2), 1.43 (s, 9H, $O(CH_3)_3$); MS (Pos FAB, HCl / glycerol) 360.11 (360.14 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

***tert*-Butyl (2*S*)-*N'*-Methyl-γ -glutamylhydrazide (46)** [247]

The method of Rueppel[247] was followed. Triethylamine (0.78 mL, 5.59 mmol) and ethyl chloroformate (1.05 mL, 11.0 mmol) were added to a cooled (-10 °C) solution of **45** in dry toluene (75 mL). The mixture was stirred for 1.5 h before **43** (2.00 g, 11.1 mmol) was added. The solution was stirred 5 min and then was stirred at room temperature for 18 h. Toluene (50 mL) was added and the mixture was washed with 5% H_3PO_4 (325 mL)

and water (2 x 100 mL) until the pH was neutral. The organic phase was dried over K_2CO_3 and concentrated *in vacuo* to give a yellow foam. Flash chromatography (50% EtOAc / 50% hexanes) yielded 2.60 (51%) of an oil: $[\alpha]_D$= +4.6 (c=3.2, CH_2Cl_2); IR (CH_2Cl_2 cast) 3300, 2978, 1725, 1241, 1155 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.60-7.44 (br s, 1H, NH), 7.31 (s, 5H, ArH), 7.28 (s, 5H, ArH), 5.64 (br d, 1H, *J*=14.0 Hz, NH), 5.14 (s, 2H, CH_2Ar), 5.04 (s, 2H, CH_2Ar), 4.24-4.05 (m, 1H, CHNH), 3.06 (s, 3H, NCH_3), 2.62-1.70 (m, 4H, CH_2CH_2), 1.41 (s, 9H, CH_3); exact mass 499.2294 (499 2318 calcd for $C_{26}H_{33}N_3O_7$). Purity of the material was determined to be greater than 95% by ^1H NMR.

N-Carbobenzyloxy-N'-Methyl-γ-glutamylhydrazide (47) [247]

The procedure of Rueppel *et al.*[247] was followed. A solution of **46** (0.614 g, 1.23 mmol) in cold trifluoroacetic acid (20 mL) was stirred at room temperature for 1 h before the trifluoroacetic acid was removed *in vacuo*. The residue was dissolved in benzene (10 mL) and the solvent was evaporated *in vacuo*. This was repeated twice to give 0.483 g (88%) of an oil: $[\alpha]_D$= +7.5 ° (c=1.6, CH_2Cl_2); IR ($CHCl_3$ cast) 3300, 1718, 1662, 1522, 1241, 1215 cm^{-1} ; ^1H NMR (200 MHz, $CDCl_3$) δ 8.1-7.5 (br m, 2H NH, CO_2H), 7.43-7.20 (m, 10H, ArH), 6.0 (br s, 1H, NH), 5.15 (s, 2H, CH_2Ar), 5.05 (s, 2H, CH_2Ar), 4.36 (br s 1H, CHNH), 3.20 (s, 3H, CH_3N), 2.7-1.7 (m, 4H, CH_2CH_2); exact mass 443.1691 (443.1692 calcd for $C_{22}H_{25}N_3O_7$). Purity of the material was determined to be greater than 85% by ^1H NMR.

N'-Methyl-γ -glutamylhydrazide (7)[247]

The procedure of Rueppel *et al.*[247] was used. A mixture of **47** (0.180 g, 0.405 mmol) in MeOH (40 mL), AcOH (0.40 mL), and 5% Pd on carbon (0.0362 g) was stirred under H_2 (1 atm) at room temperature for 50 min. This mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to an oil, which was dissolved in MeOH (5 mL) and

precipitated with ether (75 mL) to give 0.0500 g (70%) of a solid: $[\alpha]_D$= +0.87 ° (c=0.35, H_2O); IR (KBr disk) 3425, 3040, 2950, 1607, 1583, 1517, 1410 cm^{-1}; ^1H NMR (300 MHz, D_2O + TFA) δ 3.79 (dd, 1H,*J*=6.6, 6.6 Hz, CHNH), 3.04 (s, 3H, $0CH_3$), 2.45 (br t, 2H, *J*=7 0 Hz, CH_2), 2.03-1.82 (m, 2H, CH_2); MS (Pos FAB, glycerol, HCO_2H) 176.08 (176.10 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

(2*RS*, 5*R*)-5-Methyl-2-(1-methyl-1-phenylethyl)cyclohexanones 50a and 50b

The procedure of Ort[319] was modified. Phenylmagnesium bromide in ether (3M, 105 mL) was added over 1 h to a cooled (-23° C) suspension of cuprous bromide dimethyl sulfide complex (4.20 g, 20.4 mmol) in dry ether (70 mL). The dark green solution was stirred for a further 0.5 h before 96% (R)-(+)-pulegone(**49**) (40.2 g, 254 mmol) in dry ether (50 mL) was added dropwise over 1.75 h. The mixture was stirred an additional 3.75 h at -23°C and was then added to ice-cold 2M HCl (300 mL). The organic and aqueous layers were separated and the organic portion was filtered. The grey precipitate was washed with ether (2 x 200 mL) to give a yellow filtrate. The aqueous portion was saturated with NH_4Cl and was extracted with ether (3 x 100 mL). The combined organic layers were washed with saturated $KHCO_3$ (3 x 100 mL) and the solvent was removed *in vacuo* to give 62.7 g of a cloudy yellow oil. The crude ketone mixture was dissolved in a solution of KOH (70.0 g, 1.25 mol), EtOH (600 mL), and water (80 mL) and was heated to reflux for 3 h. The mixture was concentrated *in vacuo* to about 200 mL and water (500 mL) was added. The aqueous solution was saturated with NaCl and extracted with ether (5 x 100 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed *in vacuo* to give a brown oily liquid. Vacuum distillation yielded 48.0 g (82%) of a colorless oil: bp 122-126 °C at 0.8 mm Hg (lit.[319] bp 110-110 °C at 0.05 mm Hg); IR ($CHCl_3$ cast) 2950, 2920, 2863, 1710 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.37-7.21 (m,

4H, ArH), 7.20-7.12 (m, 1H, ArH), 2.72-2.67 (m, 0.85H, *trans* $CHC(CH_3)_2$), 2.50-2.43 (m, 0.15H, *cis* $CHC(CH_3)_2$), 2.28-2.20 (m, 0.85H, *trans* CHCO), 2.08-1.98 (m, 0.15H, CHCO)1.91-1.53 (m, 3H, CH_2,CH_aH),1.52-1.36 (m, 1H, CHH_b),1.46 (s, 3H, CH_3),1.42 (s, 0.45H, *cis* CH_3), 1.40 (s, 2.55H, *trans* CH_3), 1.32-1.12 (m, 1H, $CHCH_3$)0.96 (d, *J*= 6.3 Hz, 3H, *trans* CH_3) 0.89 (d, *J*=6.8 Hz, 3H, *cis* CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$) δ 211.5 (*cis* CO), 210.6 (*trans* CO), 149.6 (*trans* Ar), 149.1 (*cis* Ar), 127.8 (*cis* Ar), 127.7(*trans* Ar), 125.4 (*cis* Ar), 125.3 (*trans* Ar), 125.2 (Ar), 59.3 (*cis* CHCO), 59.1 (*trans* CHCO), 52.0 (*trans* CH_2CO), 49.9 (*cis* CH_2CO), 39.1 (*cis* $C(CH_3)_2$) 38.7 (*trans* $C(CH_3)_2$), 35.9 (*trans* CH), 34.3 (*trans* CH_2), 32.4 (*cis* CH), 31.7 (*cis* CH_2), 30.9 (*trans* CH_2), 28.7 (*trans* CH_2), 27.0 (*cis* CH_3), 26.3 (*trans* CH_3), 24.5 (*cis* CH_2), 23.6 (*cis* CH_3), 23.5(*trans* CH_3), 22.0 (*trans* CH_3), 18.9(*cis* CH_3) ratio *cis*.: *trans* 15 : 85; exact mass 230.1669 (230.1671 calcd for $C_{16}H_{22}O$). Purity of the mixture of isomers was determined to be greater than 95% by ^1H NMR.

(1*RS*, 2*SR*, 5*R*)-5-Methyl-2-(1-methyl-1-phenylethyl)cyclohexanols 51a and 51b

The method of Ort [319] was followed. A solution of **50a** and **50b** (46.7 g, 203 mmol) in iPrOH (44 mL) was added dropwise over 1h to a refluxing solution of Na (13.9 g, 604 mmol) in dry toluene (220 mL). The mixture was heated to reflux an additional 4.5 h, and was then cooled to 0 °C. Ether (275 mL) was added and the solution was filtered through glass wool into ice water (300 mL). The organic phase phase was separated, and the aqueous phase was saturated with NaCl before it was extracted with ether (3 x 100 mL). The organic layers were washed with brine (200 mL) and were dried over $MgSO_4$. Concentration *in vacuo* and vacuum distillation of the resultant oil yielded 40.1 g (85%) of a colorless oil: bp 123-126 °C at 0.5 mm Hg (lit.[319] bp 103-107 °C at 0.01 mm Hg); IR ($CHCl_3$ cast) 3560, 3420, 2951, 2919 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.34-7.26 (m, 4H, ArH), 7.21-7.14 (m, 1H, ArH), 3.76 (ddd, 0.2H, *J*= 9.8, 9.8, 4.3, *cis* CHOH),

3.52 (ddd, 0.8H, *J*= 11, 9.6, 4.3, *trans* CHOH), 2.04-1.96 (m , 0.2H, *cis* $CHC(CH_3)_2$), 1.88-1.78 (m , 0.8H, *trans* $CHC(CH_3)_2$), 1.78-1.22 (m, 5H, 2 CH_2, OH), 1.42 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.11-0.76 (m, 3H, CH_2,$CHCH_3$), 0.87 (d, *J*= 6.1 Hz, $CHCH_3$); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 151.4 (*trans* Ar), 150.9(*cis* Ar), 128.2(Ar), 125.7(Ar), 125.5(Ar), 72.7 (CHOH), 54.6(*cis* $CH(CH_3)_2Ar$), 53.8(*trans* $CH(CH_3)_2Ar$),45.3 (CH_2), 39.9 (*cis* $C(CH_3)_2$), 39.7 (*trans*$C(CH_3)_2$), 34.7 (CH_2), 31.2 (CH), 28.0 (CH_2), 26.4 (CH_3), 25.1 (*cis* CH_3), 24.7 (*trans* CH_3), 21.9 (*trans* CH_3), 21.2 (*cis* CH_3), ratio *cis /trans* 17:83; exact mass 232.1824 (232.1827 calcd for $C_{16}H_{24}O$). Anal. Calcd for.$C_{16}H_{24}O$: C, 82.70; H, 10.41. Found: C, 82.69; H, 10.43.

(1*R*, 2*S*, 5*R*)-5-Methyl-2-(1-methyl-1-phenylethyl)cyclohexyl chloroacetate (52)

The method of Ort was followed.[319] Chloroacetyl chloride (8.60 mL, 108.0 mmol) in ether (30.0 mL) was added dropwise over 45 min to a stirred, cooled (0-4 °C) solution of **51a** and **51b** (23.0 g, 99.0 mmol) and *N,N*-dimethylaniline (12.6 mL, 99.4 mmol) in ether (30.0 mL) The mixture was stirred for an additional 1 h and was then heated to reflux for 3 h. The ether was removed *in vacuo*, and the residue was partitioned between water (60 mL) and CH_2Cl_2 (60 mL). The organic layer was washed with water (60 mL) and saturated $KHCO_3$ (2 x 60 mL). The organic portion was concentrated *in vacuo* to a cloudy yellow oil which crystallized upon addition of 90% EtOH (30 mL), to yield 28.4 g (93%) of off-white crystals. Recrystallization from 95% EtOH afforded 20.2 g (66%) white needles, mp 80.5-81.5 °C (lit. [319] mp 82-83 °C); $[\alpha]_D$= +21.3° (c=1.1, CCl_4) (lit. [319] $[\alpha]_D$= +22.4° (c=2.3, CCl_4), isomeric ratio R: 97.3%, S: 2.7%; IR ($CHCl_3$ cast) 2957, 2925, 1756, 1309, 1190 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.32-7.26 (m, 4H, ArH), 7.18-7.10 (m, 1H, ArH), 4.86 (dt, J=10.8, 10.8, 4.4 Hz, 1H, CHOR), 3.17 (d, 1H, *J*=15.0 Hz, CHCl), 3.08 (d, 1H, *J*=15.0 Hz, CHCl), 2.08 (m, 1H, $CHC(CH_3)_2$), 1.93-1.78 (m, 2H, CH_aH_bCHOR), 1.74-1.63 (m, 1H, $CH_aHCHC(CH_3)_2$), 1.56-1.38 (m,

1H, $CHH_bCHC(CH_3)_2$), 1.29 (s,3H, CH_3), 1.26-1.10 (m, 1H, $CHCH_3$), 1.18 (s, 3H, CH_3), 1.04-0.84 (m, 2H, CH_2), 0.88 (d, 3H, *J*=7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6 (C=O), 151.8 (Ar), 128.0 (Ar), 125.3 (Ar), 125.2 (Ar), 75.9 (CHOR), 50.3 ($CHC(CH_3)_2$), 41.5 (CH_2Cl), 40.8 (CH_2CHOR), 39.5 ($C(CH_3)_2$), 34.5 (CH_2), 31.3 ($CHCH_3$), 29.8 (CH_3), 26.3 (CH_2), 22.8 (CH_3), 21.8 (CH_3); exact mass 308.1545 (308.1543 calcd for $C_{18}H_{25}ClO_2$) Anal. Calcd for $C_{18}H_{25}ClO_2$: C, 70.00; H, 8.16; Cl, 11.48. Found C, 70.06; H, 8.02; Cl, 11.26.

(1*R*,2*S*,5*R*)-5-Methyl-2-(1methyl-1-phenylethyl)cyclohexanol (53)

The procedure of Ort[319] was followed. A solution of **52** (13.2 g, 42.6 mmol), KOH (4.72 g, 84.3 mmol), EtOH (300 mL) and water (40 mL) was heated to reflux for 2.75 h. The solution was cooled to room temperature, concentrated *in vacuo* to 50-75 mL, and partitioned between water (200 mL) and ether (100 mL). The ether layer was removed and the aqueous layer was saturated with NaCl and extracted with ether (3x50 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo* to yield 9.78 g (99%) of a faintly yellow oil. Vacuum distillation of a 9.34 g portion afforded 8.67 g (93%) of a viscous oil: bp 115-122 °C at 0.1 mm Hg (lit. [319]bp 105-115 °C at 0.01 mm Hg); $[\alpha]_D$= -25.4 ° (c=2.1, EtOH) (lit.[319] $[\alpha]_D^{20}$= -26.4 ° (c=2.0, EtOH)) ;IR ($CHCl_3$ cast) 3560, 3400, 2950, 2918, 701 cm^{-1};^1H NMR (360 MHz, $CDCl_3$) δ 7.43-7.12 (m, 5H, ArH), 3.48 (ddd, 1H, *J*= 10.4 10.4 4.3 Hz, CHOH), 1.86-1.74 (m, 1H, CHAr), 1.74-1.53 (m, 3H, CH_2,CH_aH), 1.41 (s, 3H, CH_3), 1.36 (m, 1H, CHH_b), 1.29 (s, 3H, CH_3), 1.10-0.76 (m, 3H, CH_2, $CHCH_3$), 0.87 (d, 3H, *J*=6.1 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ151.2 (Ar), 128.3, (Ar), 128.1 (Ar), 125.7 (Ar), 125.6 (Ar), 72.6 (CHOH), 54.1 (CHAr), 45.3 (CH_2CHOH), 39.7 ($C(CH_3)_2$), 34.8 (CH_2), 31.4 ($CHCH_3$), 28.4 (CH_3), 26.4 (CH_2), 24.5 (CH_3), 21.9 (CH_3); exact mass 232.1825 (232. 1826 calcd for $C_{16}H_{24}O$). Anal. Calcd for $C_{16}H_{24}O$ C: 82.70; H: 10.41. Found C: 82.78; H: 10.46.

(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl Acrylate (54) The procedure of Whitesell *et al.*[252] was modified. Acryloyl chloride (98%, 2.00 mL 19.9 mmol) was slowly added to a cooled (0 °C) stirred solution of **53** (2.01 g, 8.65 mmol), 4-N,N'-dimethylaminopyridine (0.148 g, 1.21 mmol), and pyridine (3.00 mL, 17.8 mmol) in CH_2Cl_2 (11.0 mL). The mixture was stirred for a total of 2.75 h. Water (5 mL) was added, and the organic and aqueous phases were separated. The aqueous layer was extracted with CH_2Cl_2 (5 x 5 mL), and the organic layers were combined and concentrated. The residue was then partitioned between water and ether, and the ether layer was separated. The aqueous phase was extracted with ether (3 x 5 mL), the ether layers were combined and concentrated to give 2.40 g (97%) of an orange oil. Flash chromatography (5% EtOAc / 95% petroleum ether) on a 4.5 cm x 20 cm column afforded 1.66 g (67%) of an oil: $[\alpha]_D$= -12.2 ° (c=2.2, $CHCl_3$); IR ($CHCl_3$ cast) 2954, 2923, 1720, 1405, 1296, 1271, 1201, 1049 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.36-7.20 (m, 4H, ArH), 7.20-7.06 (m, 1H, Ar H), 6.12-5.95 (m, 1H,CH=), 5.69-5.48 (m, 2H, CH_2=), 4.88 (ddd, 1H, *J*=10.6, 10.6, 4.3 Hz, CHOR), 2.16-1.84 (m, 2H, $CHC(CH_3)_2$, CH_aHCHOR), 1.78-0.76 (m, 6H, CHH_bCHOR,CH_2CH_2, $CHCH_3$), 1.32 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 0.85 (d, 3H, *J*=6.3 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (CO_2R), 151.5 (Ar), 129.6 (CH_2=), 128.9 (CH=), 128.0 (Ar), 125.4 (Ar), 125.0 (Ar),74.6 (CHOR), 50.6 ($CHC(CH_3)_2$), 41.7 (CH_2), 39.7 ($C(CH_3)_2$), 34.6 (CH_2), 31.3 ($CHCH_3$), 27.5 (CH_3), 26.7 (CH_2), 25.4 (CH_3),21.8 (CH_3); exact mass 286.1925 (286.1933 calcd for $C_{19}H_{26}O_2$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl Oxoacetate (48) From 54

The method of Whitesell *et al.*[252] was followed. A stream of ozone (about 15%) in oxygen was passed for 10 min through a cooled (-78 °C) solution of **54** (0.569 g, 1.99 mmol) in MeOH (3.2 mL) and CH_2Cl_2 (5.1 mL), at which point the solution was faintly blue and the emerging gas from the reaction removed the color from a solution of iodine in acetic acid. Dimethyl sulfide (1.0mL, 13.6 mmol) was added and the flask was flushed with oxygen for 10 min. The reaction mixture was transferred to a -23 °C bath and was left for 24 h. The solution was concentrated *in vacuo*, and the resulting oil was dissolved in ether and washed with saturated $KHCO_3$ (2 x 5 mL) and water (5 mL). The combined ether layers were concentrated *in vacuo* and the remaining oil was heated under vacuum (95 °C, 1.4 mm Hg) for 4 h, to yield 0.438 g (76%) of a viscous oil: IR ($CHCl_3$ cast) 3460, 2954, 2923, 1741, 1222, 1092, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.38 (s, 1H, CHO), 7.34-7.14 (m, 4H, ArH), 7.13-7.07 (m, 1H, ArH), 4.68 (ddd, 1H, *J*= 11.0, 11.0, 4.3 Hz, CHOR), 2.18 (ddd, 1H, *J*=12.2, 11.0, 3.7 Hz, $CHC(CH_3)_2$), 1.96 (ddd, 1H, *J*=13.4, 11.0, 3.7 Hz, CH_aHCHOR), 1.88-1.78 (m, 1H, CHH_bCHOR) 1.78-1.71 (m, 1H, $CH_aHCHC(CH_3)_2$), 1.59-1.41 (m, 1H, $CHH_bCHC(CH_3)_2$), 1.40-0.78 (m, 3 H,CH_2CHCH_3), 1.32 (s, 3H, CCH_3), 1.29 (s, 3H, CCH_3),0.91 (d, 3H, *J*=6.1 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.8 (CHO), 157.8 (CO_2), 151.3 (Ar), 128.0 (Ar), 125.5 (Ar), 125.4 (Ar), 76.5 (CHOR), 50.5 (CHCHOR), 41.3 (CH_2CHOR), 39.4 ($C(CH_3)_2$), 34.3 (CH_2), 31.3 ($CHCH_3$), 29.8 (CCH_3), 26.1 (CH_2), 22.4 (CH_3), 21.7 (CH_3); exact mass 288.1722 (288.1725 calcd for $C_{18}H_{24}O_3$). Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found C, 74.81; H, 8.44.

(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl Oxoacetate (48) From 56

The method of Whitesell *et al.*[252] was followed. Sodium acetate trihydrate (1.17 g, 8.60 mmol) was added to a solution of **56** (2.71 g, 8.08 mmol) in DMSO (25 mL), and the suspension was stirred for 70 min. The mixture was poured into ice/water (100 mL), and the milky solution was saturated with NaCl. The mixture was extracted with ether (3 x 125 mL) and the combined extracts were washed with saturated $NaHCO_3$ (2 x 75 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give an off-white amorphous solid. Distillation *in vacuo* gave 1.95 g (97%) of a pale yellow oil: bp 155-163 °C at 0.6 mm Hg (lit.[252] bp 135-142 °C at 0.1 mm Hg); $[\alpha]_D$= -1.30 ° (c=5.7, $CHCl_3$); the remaining spectral data were identical to those of **48** obtained from **54**.

(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl Bromoacetate (55)

The procedure of Whitesell *et al.*[252] was employed. *p*-Toluenesulfonic acid monhydrate (0.334 g, mmol) was added to a solution of **53** (3.13 g, 13.5 mmol) and bromoacetic acid (5.14 g, 37.0 mmol) in benzene and heated to reflux with the removal of water for 6 h. The mixture was cooled and poured into 2M Na_2CO_3 (40 mL). The organic layer was removed, and the aqueous layer was extracted with ether (3 x 50 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to 4.30 g (90%) of a yellow oil. A 0.450 g portion was removed and purified by flash chromatography (hexanes, then EtOAc) to give 0.365 g (81%) of an oil: $[\alpha]_D$= +21.4 ° (c=2.5, $CHCl_3$); IR (CH_2Cl_2 cast) 2923, 1746, 1731, 1291, 1281, 1129 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.32-7.21 (m, 4H, ArH), 7.16-7.08 (m, 1H, ArH), 5.85 (ddd, 1H, *J*= 11.0, 10.4, 4.3 Hz, CHOR), 3.02 (d, 1H, *J*=12.8 Hz, CH_aHBr), 2.93 (d, 1H, *J*=12.8 Hz, CHH_bBr), 2.09-1.99 (m, 1H, $CHC(CH_3)_2$), 1.88-1.76 (m, 2H, CH_aHCHOR, $CH_aHCHC(CH_3)_2$), 1.71-1.60 (m, 1H, CHH_bCHOR), 1.50-1.45 (m, 1H, $CHH_bCHC(CH_3)_2$), 1.30 (s, 3H, CCH_3), 1.20-

1.05(m, 1H, $CHCH_3$),1.19 (s, 3H, CCH_3), 1.00-0.87 (m, 2H, CH_2), 0.87 (d, 3H, J=6.7 Hz, $CHCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.3 (CO_2), 151.6 (Ar), 127.9 (Ar), 125.3 (Ar), 125.1 (Ar), 75.9 (CHOR), 50.3 (CHCHOR), 41.2 (CH_2CHOR), 39.4 ($C(CH_3)_2$), 34.4 (CH_2), 31 2 ($CHCH_3$), 29.4 (CCH_3), 26.3 (CH_2), 26.2 (CH_2), 23.0 (CH_3), 21.7 (CH_3) exact mass 352.1043 (352.1038 calcd for $C_{18}H_{25}BrO_2$). Anal. Calcd for $C_{18}H_{25}BrO_2$: C, 61.19; H, 7.13; Br, 22.62. Found: C, 61.59; H, 6.82; Br, 23.15.

(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl Nitrooxyacetate (56)

The method of Whitesell and coworkers[252] was modified. Silver nitrate (3.53 g, 20.8 mmol) was added to a solution of **55** (3.65 g, 10.3 mmol) in CH_3CN (50 mL), and the mixture was stirred at room temperature for 48 h. Additional silver nitrate (1.05 g, 6.18 mmol) was added and the reaction was stirred a further 48 h. The solvent was removed *in vacuo* and the residue was extracted with ether. The combined ether layers were washed with water (3 x 40 mL) and dried over Na_2SO_4. Concentration *in vacuo* yielded a yellow oil which was purified by flash chromatography (hexanes, followed by 20% EtOAc / 80% hexanes) to give 3.02 g (87%) of an oil: $[\alpha]_D$= +21.7 ° (c=0.91, $CHCl_3$); IR (CH_2Cl_2 cast) 2956, 1751, 1655, 1292, 1220 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.38-7.18 (m, 4H, ArH), 7.18-7.07 (m, 1H, ArH), 5.90 (ddd, 1H, J= 11.0, 10.4, 4.3 Hz, CHOR), 5.19 (d, 1H, J=17.1 Hz, CH_aHONO_2), 4.78 (d, 1H, J=17.1 Hz, CHH_bONO_2), 2.14-2.00 (m, 1H, $CHC(CH_3)_2$), 1.94-1.76 (m, 1H, CH_aHCHOR), 1.88-1.78 (m, 2H, CHH_bCHOR, $CH_aHCHC(CH_3)_2$) 1.73-1.61 (m, 1H, $CHH_bCHC(CH_3)_2$), 1.52-1.37 (m, 1H, CH_aH), 1.37-0.87 (m, 2H, CHH_b, CH_2CHCH_3), 1.30 (s, 3H, CCH_3), 1.19 (s, 3H, CCH_3),0.89 (d, 3H, J=6.7 Hz, $CHCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.9 (CO_2), 151.6 (Ar), 128.0 (Ar), 125.3 (Ar), 125.0 (Ar), 75.8 (CHOR), 66.7 (CH_2ONO_2), 50.0 (CHCHOR), 41.4 (CH_2CHOR), 39.3 ($C(CH_3)_2$), 34.3 (CH_2), 31 2 ($CHCH_3$), 29.8 (CCH_3), 26.1 (CH_2), 22.5 (CH_3), 21.6 (CH_3); exact mass 335.1732

(335.1733 calcd for $C_{18}H_{25}NO_5$). Anal. Calcd for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found C, 64.08; H, 7.39; N, 3.87.

2-Acetamido-4-pentenoic Acid (58)

The procedure of Black and Wright [323] was used. Acetic anhydride (65.0 mL, 688 mmol) was added in five portions to a stirred, cooled (0 °C) solution of D,L-allylglycine (**57**) (50.9 g, 442 mmol) in 2 M NaOH (225 mL) over 1 h. After each addition the pH was readjusted to above pH 9 with saturated NaOH. After all the acetic anhydride was added, the solution was stirred a further 20 min before concentrated HCl (130 mL) was added. The mixture was extracted with EtOAc (5 x 400 mL), and the combined organic phases were dried over Na_2SO_4. Concentration *in vacuo* yielded 69.8 g of a colorless solid. Recrystallization in acetone/ petroleum ether afforded 66.7 g (96%) of a white powder: mp 114-115 °C (lit.[323] mp 114 °C) IR (CH_3OH cast) 3400-2800, 1724 ,1617, 1541, 1230, 1201 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 5.85-5.72 (m, 1H, CH=), 5.18-5.05 (m, 2H, CH_2=), 4.44 (dd, *J*=8.5, 4.9 Hz, 1H, $CHCO_2R$), 2.64-2.54 (m, 1H, CH_aHCHCO_2R), 2.48-2.34 (m, 1H, CHH_bCHCO_2R), 1.97 (s, 3H, CH_3); exact mass 157.0739 (157.0738 calcd for $C_7H_{11}NO_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(2*S*)-2-Amino-4-pentenoic Acid (60)

A solution of **58** (35.8 g, 0.228 mol) in water (1800 mL) was adjusted to pH 7.9 with $LiOH{\cdot}2H_2O$ and AcOH. Hog Kidney Acylase I (0.0460 g) was added and the solution agitated at 37 °C for 23 h. The solution was cooled, the pH was adjusted to pH 4.9, and Norit (6.75 g) was added. The mixture was filtered and the filtrate was concentrated *in vacuo* until a precipitate began to form. EtOH (400 mL) was added and the mixture was left to crystallize, yielding 7.25 g colorless crystals. Concentration and crystallization of the mother liquor gave 2.99 g colorless crystals. Recrystallization from water / EtOH

afforded 7.20 g (55%) of colorless flakes: $[\alpha]_D$= -37.8 ° (c=2.5, water) (lit.[323] $[\alpha]_D^{24}$= -37.1° (c= 4%, water)); IR (KBr disk) 3420, 2900, 1643, 1612, 1586, 1561, 1512, 1405 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 5.86-5.72 (m, 1H, CH=), 5.36-5.22 (m, 2H, CH_2=), 3.83 (dd, 1H, *J*= 7.0, 5.2 Hz, CHNH), 2.74-2.55 (m, 2H, CH_2); MS (Pos FAB , glycerol HCO_2H) 116.14 (116.06 calcd for MH^+). Anal. Calcd for $C_5H_9NO_2$: C, 52.17; H, 7.88 ; N, 12.17 . Found C, 52.12 ; H, 7.73 ; N, 12.23.

(2*R*)-2-Acetamido-4-pentenoic Acid (59)

The procedure of Black and Wright[323] was followed. The filtrate from the crystallization of **60** was concentrated *in vacuo* and dissolved in water (100 mL). Acidification to pH 1 with concd HCl, followed by extraction with EtOAc (4 x 250 mL), drying of the organic extracts over Na_2SO_4, and evaporation of the solvent *in vacuo* yielded a colorless solid. Recrystallization from acetone / petroleum ether gave 11.2 g (63%) colorless crystals: mp 104.5-105 °C ; $[\alpha]_D$= -42.7 ° (c=4.1, EtOH) (lit.[323] $[\alpha]_D^{22}$= -42.0 ° (c=4 %, EtOH)); IR (KBr disk) 3332, 1702, 1626, 1558, 1240 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 6.7-6.56 (m, 1H, CH=), 6.08-5.92 (m, 2H, CH_2=), 4.24 (dd, *J*= 8.0, 5.0 Hz, 1H, CHNH), 2.48-2.26 (m, 2H, CH_2), 1.84 (s, 3H, CH_3); exact mass 157.0740 (157.0739 calcd for $C_7H_{11}NO_3$). Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found C, 53.55; H, 6.89; N, 9.00.

(2*R*)-2-Amino-4-pentenoic Acid (61)

The method of Black and Wright[323] was used. A solution of **59** (11.24 g, 71.5 mmol) in 2M HCl (150mL) was heated to reflux for 2.5 h. The solution was cooled and the pH adjusted to pH 4.5-5 with 4N LiOH. The mixture was concentrated *in vacuo* to about 75 mL, and EtOH (400 mL) was added. The resultant crystals were filtered and dried to give 5.15 g (63%) of colorless needles: $[\alpha]_D$= +35.2 ° (c=4.2, water). A 1.05 g portion was recrystallized from water / EtOH to give 0.670 g (65%) of colorless plates: $[\alpha]_D$= +38.2 °

(c=2.5, water) (lit.[323] $[\alpha]_D$= +37.8 ° (4%, water); IR (KBr disk) 3430, 2930, 1616, 1587, 1512 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 5.86-5.72 (m, 1H, CH=), 5.36-5.22 (m, 2H, CH_2=), 3.83 (dd, 1H, *J*= 7.0, 5.2 Hz, CHNH), 2.74-2.55 (m, 2H, CH_2); MS (Pos FAB, glycerol) 116.22 (116.06 calcd for MH^+). Anal. Calcd for $C_5H_9NO_2$: C, 52.17; H, 7.88 ; N, 12.17 . Found: C, 52.02 ; H, 7.66 ; N, 12.09.

Methyl (2*R*)-2-([(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-carbonyl]amino)-pent-4-enoate (65) [324]

The procedure of Arnold[324] was followed. (*S*) Camphanic acid chloride (**62**) (98%, 33.1 mg, 0.150 mmol) in toluene (0.75 mL) was added to a solution of **61** (11.8 mg, 0.103 mmol) in 1.0M pH 10 $NaHCO_3$ / Na_2CO_3 buffer (3 mL), and the mixture was stirred vigorously for 2 h. The solution was acidified to pH 1 with 5.7M HCl and was then extracted with CH_2Cl_2 (4 x 3 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a colorless solid. This residue was dissolved in ether and treated with CH_2N_2 in ether until a permanent yellow color was obtained. Evaporation *in vacuo* yielded a colorless oil. Preparative thin layer chromatography (35% EtOAc / 65% hexanes) yielded 17.4 mg (55%) of a colorless oil: $[\alpha]_D$= -8.8 ° (c=1.7, $CHCl_3$); IR ($CHCl_3$ cast) 3420, 3360, 2967, 1794, 1747, 1677, 1526 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.82 (d, 1H, *J*=7.9 Hz, NH), 5.80-5.67 (m, 1H, CH=), 5.28-5.16 (m, 2H, CH_2=), 4.77-4.68 (m, 1H, CHNH), 3.78 (s, 3H, OCH_3), 2.70-2.48 (m, 3H, $CHCH_2$, CH_aHCCON), 2.04-1.86 (m, 2H, CHH_bCCON, CH_aHCCH_3), 1.76-164 (m, 1H, CHH_bCCH_3),1.12 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); exact mass 309.1576 (309.1575 calcd for $C_{16}H_{23}NO_5$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl (2*S*)-2-([(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-carbonyl]amino)-pent-4-enoate (64)

The same method[324] was used to prepare **65** from **61** (9.35 mg, 0.0812 mmol) in 48% yield as that used to convert **60** to **64**, using 39.2 mg (0.181 mmol) (*S*)-camphanic acid chloride, 3 mL buffer, and 0.75 mL toluene: $[\alpha]_D$= +1.8 ° (c=2.0, $CHCl_3$); IR ($CHCl_3$ cast) 3420, 3360, 2968, 1793, 1748, 1676, 1527 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.92 (d, 1H, *J*=7.9 Hz, NH), 5.77-5.63 (m, 1H, CH=), 5.20-5.09 (m, 2H, CH_2=), 4.79-4.69 (m, 1H, CHNH), 3.76 (s, 3H, OCH_3), 2.68-2.43 (m, 3H, $CHCH_2$, CH_aHCCON), 2.03-1.86 (m, 2H, CHH_bCCON, CH_aHCCH_3), 1.76-164 (m, 1H, CHH_bCCH_3), 1.12 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 0.91 (s, 3H, CH_3); exact mass 309.1575 (309.1575 calcd for $C_{16}H_{23}NO_5$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl (1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate (63)

This compound was obtained by sublimation *in vacuo* or by column chromatography from the camphanamide derivatives **64**, **65**, **107**, **108**, **109**, and **110** in 30-37% yield: mp 106-107 °C (lit.[325] mp 108.4-108.5 °C); $[\alpha]_D$ = -13.2 ° (c= 2.2, 95% EtOH) (lit.[325] $[\alpha]_D$ = -12.4 ° (c=2.2, 95% EtOH)); IR ($CHCl_3$ cast) 2963, 1782, 1727, 1278, 1101 cm^{-1}; ^1H NMR (360 MHz,$CDCl_3$) δ 3.84 (s, 3H, OCH_3), 2.53-2.35 (m , 1H, $CH_aHCR_2CO_2CH_3$) 2.13-1.86 (m, 2H, $CHH_bCR_2CO_2CH_3$, $CH_aHCR_2CO_2R$), 1.79-1.62 (m, 1H, $CHH_bCR_2CO_2R$), 1.12 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.9 (CO_2R), 167.9 (CO_2R), 91.1(CR_3O), 54.6 (CH_2), 54.1 (CR_4), 53.4 (OCH_3), 52.4 (CR_4), 30.6 (CH_3), 28.9 (CH_3), 16.7 (CH_3), 9.6 (CH_3); exact mass 212.1044 (212.1049 calcd for $C_{11}H_{16}O_4$). Anal. Calcd for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 62.41; H, 7.47.

GC Analysis of the Camphanoate Esters 64 and 65

GC analyses were performed on a Hewlett Packard 5890 gas chromatograph equipped with an Alltech fused silica open tubular (FSOT) megabore column (10m x 0.53 mm) RSL 300 1.2 μm inside diameter. Column conditions were as follows: injector and detector temperature 250 °C; carrier gas (helium) 7.5 psi at 150 °C, initial temperature 150 °C; initial time 2 min; rate 1 °C/ min; final temperature 200 °C; final time 2 min; rate II 50 °C/ min; final time II 2.00 min; equilibration time 2.00 min. Injections of 2.00 μL were made from solutions containing 1.3-1.8 mg/mL of the crude camphanamides in ether (Et_2O). Typical retention times determined were: 34.9 min for D-camphanamide **65**, 36.3 min for L-camphanamide **64**. Methyl camphanoate (**63**) had a retention time of 6.3 min. Ratios were measured by integration and comparison of the appropriate peaks.

(2*S*)-2-(*N*-Carbobenzyloxy)amino-4-pentenoic Acid (66)

The method of Neuberger and Tait[357] was modified. Benzyl chloroformate (95%, 13.0 mL, 86.5 mmol) was added to a cooled (0 °C) stirred solution of L-allylglycine (**60**) (4.84 g, 42.1 mmol) in 1M pH 10 $NaHCO_3$/ Na_2CO_3 buffer (200 mL) / dioxane (67.0 mL). The pH was adjusted during the reaction with saturated NaOH, and stirring was continued for 1.67 h. The cloudy solution was washed with CH_2Cl_2 (2 x 100 mL), and the aqueous layer was acidified to pH 1 with 5.7 M HCl before extraction with EtOAc (4 x 200 mL). The combined EtOAc extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give 10.3 g (99% yield) of a thick oil. Recrystallization from ether/hexanes afforded 8.70 g (83% yield) of white crystals: mp 63.5-64.5 °C (lit.[357] mp 65 °C); $[\alpha]_D$= +19.4 ° (c=0.41, $CHCl_3$,) (lit.[357] $[\alpha]_D$= +17.6 ° (c=5.0, $CHCl_3$)); IR ($CHCl_3$ cast) 3320 br, 1718 br, 1521, 1260, 1217, 1055 cm^{-1}; ^1H NMR (360 MHz $CDCl_3$) δ 11.87 (br s, 1H, CO_2H), 7.44-7.31 (m, 5H Ar), 5.82-5.68 (m, 1H, CH=), 5.29 (d, 1H, *J*=11.0 Hz, NH), 5.24-5.04 (m, 4H, CH_2=CH, CH_2Ar), 4.49 (dd, 1H, *J*=13.4, 6.1 Hz, CHNH), 2.70-2.46 (m, 2H, CH_2CHNH); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 176.5 (CO_2H), 155.9

(CONH), 136.6 (Ar), 131.0 (CH=), 129.2 (Ar), 127.4 (Ar), 119.7 (CH_2=), 67.2 (OCH_2), 53.1 (CH), 35.5 (CH_2); exact mass 249.1003 (249.1001 calcd for $C_{13}H_{15}NO_4$). Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.50 ; H, 6.10; N, 5.57.

(2*R*)-2-(*N*-Carbobenzyloxy)amino-4-pentenoic Acid (67)

The method used to make **66** from **60** was used to prepare **67** from **61** (3.00 g, 26.1 mmol) in 90% yield, using 8.00 mL (95%, 56.0 mmol) benzyl chloroformate in 100 mL buffer: mp 62-64 °C; $[\alpha]_D$= -13.0 ° (c=5.0, $CHCl_3$); other spectroscopic properties of **67** were identical to those of **66**.

Methyl (2*S*)-2-(*N*-Carbobenzyloxy)amino-4-pentenoate (68) [358]

Compound **66** (8.46 g, 33.9 mmol) was dissolved in a minimum amount of ether and cooled to 0 °C. Freshly prepared CH_2N_2 in ether was slowly added with stirring until a permanent yellow color was obtained. The solution was warmed to room temperature, the excess CH_2N_2 was removed *in vacuo*, and the resultant oil was purified by flash chromatography (EtOAc 30% / hexane 70%) to give 8.55 g (96% yield) of a colorless oil: $[\alpha]_D$= +16.0 ° (c=1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3360, 1742, 1725, 1524, 1264, 1216 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.45-7.22 (m, 5H, Ar), 5.75-5.61 (m, 1H, $CHCH_2$), 5.47 (br d, 1H, *J*=8 Hz, NH), 5.24-5.11 (m, 2H, $CHCH_2$), 5.09 (s, 2H, CH_2Ar), 4.44 (dd, 1H, *J*=13.5, 6.3 Hz, CHNH), 3.70 (s, 3H, OCH_3), 2.63-2.40 (m, 2H, CH_2CHNH); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 172.1 (CO_2R), 155.6 (CONH), 136.1 (Ar), 132.0 (CH=), 129.1 (Ar), 127.3 (Ar), 119.1 (CH_2=), 66.8 (OCH_2), 53.2 (CH), 52.2 (OCH_3), 36.5 (CH_2); exact mass 263.1157 (263.1157 calcd for $C_{14}H_{17}NO_4$). Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.92; H, 6.39; N, 5.24.

Methyl (2*R*)-2-(*N*-Carbobenzyloxy)amino-4-pentenoate (69)

The procedure to prepare **66** from **60** was used to make **69** from **67** (4.00 g, 16.0 mmol) in 83% yield: $[\alpha]_D$= -14.5 ° (c=4.7, $CHCl_3$); other spectral properties identical to those of **68** above.

Ethyl 2-Amino-4-pentenoate Hydrochloride (70)[359]

The method of Greenstein and Winitz[360] was followed. HCl gas was bubbled through a suspension of D,L allylglycine (1.15 g, 9.98 mmol) in dry EtOH (50 mL) until all the amino acid had dissolved. The solution was cooled (0 °C) and HCl was bubbled through for a further 15 min. The mixture was stirred for 12 h at room temperature. The solvent was removed *in vacuo* , the resultant syrup was resuspended in dry EtOH and concentrated again *in vacuo*. Suspension in ether gave 1.58 g (88%) of a colorless solid: mp 70-72 °C; IR (KBr disk) 3320, 1748, 1520, 1227 cm^{-1} ; ^1H NMR (360 MHz, $CDCl_3$) δ 5.92-5.85 (m, 1H, CH=), 5.42-5.25 (m, 2H, CH_2=), 4.35-4.16 (m, 3H, CHNH, OCH_2), 2.94-2.80 (m, 2H, CH_2CH=), 2.30 (t, 3H, *J*= 7.3 Hz, CH_3); exact mass 144.1026(144.1024 calcd for $C_7H_{14}NO_2$).MS (Pos FAB, glycerol) 143.89 (143.09 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Ethyl 2-Acetamido-4-pentenoate (71) [361]

The procedure of Greenstein and Winitz was modified.[362] Acetic anhydride (13 μL, 0.138 mmol) was added to a solution of **70** (19.3 mg, 0.107 mmol) in 0.20M NaOH (0.50 mL). NaOH (2M, 60 μL) was added, and the mixture was stirred at room temperature for 2 h. The solution was extracted with EtOAc (3 x 5 mL) and the extracts were dried over Na_2SO_4. Concentration *in vacuo* gave 13.3 mg (67%) of **71** as a colorless oil. Acidification of the aqueous phase, extraction with EtOAc (3 x 5 mL), dessication over Na_2SO_4, and concentration *in vacuo* afforded 3.98 mg (24%) of the starting amine **70**. For **71**: IR ($CHCl_3$ cast) 3282, 1744, 1657, 1544, 1375, 1195 cm^{-1}; ^1H NMR (360 MHz,

$CDCl_3$) δ 6.13 (br d, 1H, *J*=26.8 Hz, NH), 5.75-5.62 (m, 1H, CH=), 5.16-5.08 (m, 2H, CH_2=), 4.67 (dd, 1H, *J*=13.7,6.1 Hz, $CHCO_2$), 4.40-4.14 (m, 2H, CH_2CH_3), 2.65-2.46 (m, 2H, CH_2CHCO_2), 2.04 (s, 3H, CH_3), 1.29 (t, 3H, *J*=7 Hz, CH_3); exact mass 185.1054 (185.1052 calcd for $C_9H_{15}NO_3$). Purity of the material was determined to be greater than 95% by ^{1}H NMR.

1-Ethyl 7-(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl (2*RS*, 6*S*)-2-Acetamido-6-hydroxy-3-heptenedioate (72)

Stannic chloride (52 μL, 0.451 mmol) was added to a cold (- 78 °C) solution of **48** (0.0431 g, 0.149 mmol) in dry CH_2Cl_2 (1.5 mL). The solution was stirred for 12 min before **71** (0.0139 g, 0.0749 mmol) was added in dry CH_2Cl_2 (1.0 mL). The mixture was stirred for 2 h and then was left at -10 °C for 16.5 h. The reaction was quenched with ether (3 mL) and was washed with saturated $KHCO_3$ (2 x 4 mL) and water (4 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give an amorphous solid. Flash chromatography (10% hexanes / 90% EtOAc) yielded 0.0201 g (57%) of an oil: IR ($CDCl_3$ cast) 3320, 2957, 1738, 1657, 1371, 1259, 1241, 1201, 1111, 1094 cm^{-1}; ^{1}H NMR δ 7.36-7.25 (m, 4H, ArH), 7.18-7.10 (m, 1H, ArH) 6.10 (br d, 1H, *J*= 7.3 Hz, NH), 5.64-5.47 (m, 2H, CH=CH), 5.01 (dd, 1H, *J*= 7.5, 4.5 Hz, CHNH), 4.85 (ddd, 1H, *J*= 10.5, 10.5 4.4 Hz, CHOR), 4.28-4.19 (m, 2H, OCH_2), 3.27-3.18 (m, 1H, CHOH), 2.62-2.55 (m, 1H, $CHC(CH_3)_2$), 2.20-1.40 (m, 7H, 3 CH_2, OH), 2.04 (s, 3H, $COCH_3$), 1.34-1.10 (m, 4 H, $CHCH_3$, CH_3), 1.26 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.02-0.84 (m, 2H, CH_2), 0.90 (d, 3H, *J*= 6.1 Hz, CH_3); ^{13}C NMR (MHz, $CDCl_3$), δ 173.4 (CO_2R), 170.7 (CO_2R), 169.4 (CO_2R), 151.8 (CONH), 132.2 (Ar), 128.4 (Ar), 128.1 (Ar), 127.9 (Ar), 127.6 (Ar), 125.2 (CH=), 76.0 (CHOR), 69.05 (CHOH), 68.99 (CHOH), 61.7 (OCH_2), 53.8 (CHNH), 50.2 ($CHC(CH_3)_2$), 41.5 (CH_2), 39.4 ($C(CH_3)_2$), 36.5 (CH_2), 36.4 (CH_2), 34.4 (CH_2), 31.2 ($CHCH_3$), 29.5 (CH_3), 26.2 (CH_2), 23.1 (CH_3), 23.0 (CH_3), 21.8 (CH_3), 14.1 (CH_3); exact mass 473.2771

(473.2777 calcd for $C_{27}H_{39}NO_6$). Purity of the material was determined to be greater than 95% by ^1H NMR.

1-Methyl 7-(1*R*, 2*S*, 5*R*)-2-(1-Methyl-1-phenylethyl)-5-methylcyclohexyl (2*S*, 6*S*)-2-(*N*-Carbobenzyloxy)amino-6-hydroxy-3-heptenedioate (73)

A procedure analogous to that used to prepare **72** was used to generate **73** from **48** and **68** in 62% yield. $SnCl_4$ (52 μL, 0.451 mmol), **48** (0.0431 g, 0.149 mmol), and **68** (19.4 mg, 0.0738 mmol) were employed: $[\alpha]_D$= +12.3 ° (c= 2.3, $CHCl_3$); IR ($CHCl_3$ cast) 3440, 3370, 2934, 2924, 1728, 1272, 1259, 1218, 1092, 1049 cm^{-1} ; ^1H NMR (360 MHz, $CDCl_3$) δ 7.38-7.24 (m, 9H, ArH), 7.15-7.08 (m, 1H, ArH), 5.62-5.45 (m, 2H, CH=CH), 5.35 (br d, 1H, *J*= 7.9 Hz, NH), 5.11 (s, 2H, CH_2Ar), 4.93-4.78 (m, 2H, CHNH, CHOR), 3.75 (s, 3H, OCH_3), 3.28-3.15 (m, 1H, CHOH), 2.65-2.53 (m, 1H, $CHC(CH_3)_2$), 2.22-0.82 (m, 10H, 4 CH_2, $CHCH_3$, OH), 1.26 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 0.88 (d, 3H, *J*= 6.1 Hz, $CHCH_3$); ^{13}C NMR (100MHz, $CDCl_3$) δ 173.5 (CO), 171.0 (CO), 151.8 (CO), 136.2 (Ar), 128.7 (Ar), 128.5 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.4 (Ar), 125.19 (CH=), 125.16 (CH=), 76.0 (CHOR), 69.1 (CHOH), 67.1 (OCH_2), 55.5 (CHNH), 52.6 (OCH_3), 50.3 (CHCHOR), 41.6 (CH_2CHOR), 39.4 ($CCH_3)_2$), 36.5 (CH_2), 34.4 (CH_2), 31.2 ($CHCH_3$), 29.5 (CH_3), 26.2 (CH_2), 23.0 (CH_3), 21.7 (CH_3); exact mass 551.2879 (551.2883 calcd for $C_{32}H_{41}NO_7$),MS (CI NH_3) 570 (570 calcd for $C_{32}H_{41}NO_7$ +NH_4^+). Anal Calcd for $C_{32}H_{41}NO_7$: C, 69.67; H,7.49; N 2.54. Found:C, 69.46; H,7.69; N 2.29.

Cyclohexene Oxide (77)

The procedure of Godchot and Bédos[363] was modified. A solution of *meta*-chloroperbenzoic acid (70.1 g, 0.406 mol) in CH_2Cl_2 (300 mL) and ether (100 mL) was added dropwise to a cooled (0 °C) stirred solution of cyclohexene (**76**) (30.0 g, 0.366 mol) in CH_2Cl_2 (50 mL). Once the addition was complete, ether (100 mL) was added to

the slurry and the solution was stirred for 0.25 h at 0 °C, and at room temperature for 18 h. The mixture was washed with 1M NaOH (2 x 200 mL) and water (2 x 200 mL), and the organic phase was dried over Na_2SO_4. Distillation afforded 21.6 g (60%) of a colorless liquid: bp 127-128 °C 740 mm Hg (lit.[363] bp 131-132 °C); IR (neat) 2982, 2937, 2858, 1467, 1463, 1258, 838, 781 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 3.14 (d, 2H, *J*=1 Hz,, CHO), 2.08-1.90 (m, 2H, 2CHCHO), 1.90-1.75 (m, 2H, 2CHCHO), 1.53-1.38 (m, 2H, 2CH), 1.33-1.17 (m, 2H, 2CH); exact mass 98.0725 (98.0732 calcd for $C_6H_{10}O$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*RS*, 2*SR*)-*trans*-2-Phenylcyclohexanol (74)

The method of Whitesell and Lawrence[327] was adapted. A solution of bromobenzene (156 mL, 1.48 mol) in THF (300 mL) was slowly added to a suspension of magnesium turnings (35.3 g, 1.45 mol) in dry THF (200 mL). The mixture was stirred at room temperature for 50 min before additional THF (900 mL) was added and the solution was cooled to -12 °C. Purified CuCl (6.59 g, 66.6 mmol) was added, the mixture was stirred for 10 min, and cyclohexene oxide **77** (98%, 102 mL, 988 mmol) was added dropwise over 45 min. The mixture was stirred at room temperature for 2 h, then saturated $(NH_4)_2SO_4$ (500 mL) was added, and the organic phase was decanted from the resultant grey precipitate. The organic layer was washed with saturated $(NH_4)_2SO_4$ (2 x 200 mL). The combined aqueous washes were extracted with ether (2 x 300 mL) and the resulting combined organic layers were dried over $MgSO_4$. Removal of solvent *in vacuo* yielded a beige solid. Recrystallization from pentane gave 138 g (79%) of white needles: mp 56-57 °C (lit.[327] mp 56.5-57 °C); IR ($CHCl_3$ cast) 3280, 2930, 2917, 2846, 1048 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.42-7.22 (m, 5H, ArH), 3.65 (ddd, 1H, *J*=10.4, 9.8, 4.3 Hz, CHOH), 2.42 (ddd, 1H, *J*=12.2, 9.8, 3.7 Hz, CHAr), 2.16-2.09 (m, 1H, CHCHOH), 1.92-1.83 (m, 2H, CHCHAr and CHCHOH), 1.83-1.72 (m, 1H, CHCHAr), 1.63-1.26 (m, 5H, CH_2CH_2, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.3

(Ar), 128.7 (Ar), 127.9 (Ar), 126.7 (Ar),74.3 (CHOH), 53.2(CHAr), 34.4 (CH_2CHOH), 33.3 (CH_2CHAr), 26.0 (CH_2), 25.0 (CH_2); exact mass 176.1196 (176.1201 calcd for $C_{12}H_{16}O$). Anal. Calcd.for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 81.71; H, 9.21.

(1*RS*, 2*SR*)-*trans*-2-Phenylcyclohexyl Acetate (78)

The procedure of Whitesell and Lawrence[327] was used. A solution of **74** (88.0 g, 0.499 mol) in CH_2Cl_2 (125 mL)was added dropwise to a solution of 4-*N*,*N*-dimethylaminopyridine (2.10 g, 17.2 mmol) and pyridine (90.0 mL, 1.10 mol) in CH_2Cl_2 (300 mL) over 0.5 h. A solution of acetic anhydride (94.1 mL, 0.996 mol) in CH_2Cl_2 (75 mL) was added dropwise over 1.5 h. The mixture was stirred a further 1.75 h, and then was poured into a mixture of 5.7M HCl (300 mL), ice (400 mL), and ether (750 mL). The aqueous layer was removed and the organic phase was washed with 2M HCl (4 x 150 mL). The combined aqueous portions were extracted with ether (2 x 400 mL). The combined organic fractions were washed with saturated $NaHCO_3$ (2 x 200 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to give 106 g (97%) of a beige oil. Vacuum distillation (0.4mm Hg) of a 91.2 g portion afforded 88.4 g (97%) of a colorless oil: bp 104-105 °C at 0.4 mm Hg; IR($CHCl_3$ cast) 3029, 2935, 2858, 1736, 1495, 1449, 1242, 1071cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.34-7.16 (m, 5H, ArH), 4.99 (ddd, 1H, *J*=11.0, 11.0, 4.3 Hz, CHOR), 2.56 (ddd, 1H, *J*=12.2, 11.0, 3.7 Hz, CHAr), 2.18-2.06 (m, 1H, CH_aHCHOR), 2.00-1.73 (m, 3H, CH_2CHAr and CHH_bCHOR), 1.76 (s, 3H, CH_3), 1.67-1.28 (m, 4H, CH_2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1 (CO_2R), 143.0 (Ar), 128.1 (Ar), 127.4 (Ar),126.3 (Ar), 75.8 (CHOR), 49.7 (CHAr), 33.7 (CH_2CHOR), 32.3 (CH_2CHAr), 25.7 (CH_2),24.7 (CH_2), 20.8 (CH_3); exact mass 218.1301 (218.1307 calcd for $C_{14}H_{18}O_2$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Enzymatic Resolution of (1*RS*, 2*SR*)-*trans*-2-Phenylcyclohexyl Acetate to (1*R*, 2*S*)-trans-2-Phenylcyclohexanol 75 and (1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl Acetate (79)

The procedure of Whitesell and Lawrence[327] was modified. The acetate (88.4 g, 405 mmol) in acetone (445 mL) was added with vigorous stirring to 0.5M KH_2PO_4/K_2HPO_4 buffer pH 8.0 (3.24 L). The mixture was stirred for 1 h before pig liver acetone powder (PLAP) (13.5 g) was added. The mixture was stirred at room temperature for 12 days and was monitored by removal of 1 ml aliquots, which were acidified to pH 1 and extracted with CH_2Cl_2. These organic aliquots were dried over Na_2SO_4, concentrated *in vacuo*, and the residue was dissolved in $CDCl_3$. Integration of the ^1H NMR (80 MHz)spectrum and comparison of the CHOR versus CHOH signals indicated the extent of reaction. The final ratio obtained was approximately 52:48. The reaction was quenched by acidification to pH 3.5 with 2M HCl followed by addition of NaCl (400 g) and CH_2Cl_2 (250 mL). The mixture was stirred 1 h and then allowed to stand at room temperature for 3 h. The aqueous and organic phases were filtered through cotton wool and the resultant emulsion was extracted in 1.5 -2 L portions with CH_2Cl_2 (3 x 250 mL). Emulsions were broken by filtration through cotton wool. All cotton wool used in filtrations was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give 95.5 g of a brown oil. Flash chromatography (15% EtOAc / 85% hexanes) on a 5 cm x 30 cm column followed by repeated flash chromatography (20% EtOAc / 80% hexanes) on a 32.5 cm x 10 cm column yielded 31.3 g (90%) of the alcohol and 42.2 g (quant.) of the acetate **79**. Repeated recrystallization of the alcohol from distilled pentane afforded 17.4 g (56%) of the optically pure alcohol **75**: mp 64-65 °C (lit.[327] mp 64-65 °C) $[\alpha]_D$= -59.0 ° (c=10, MeOH) (lit. $^{327}[\alpha]_D^{27}$= -58.4 ° (c=10, MeOH)) IR ($CHCl_3$ cast) 3290, 2937, 2927, 2920, 1445, 1061, 1051 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) ^1H NMR (360 MHz, $CDCl_3$) δ 7.42-7.22 (m, 5H, ArH), 3.65 (m, 1H, CHOH), 2.42 (ddd, 1H, *J*=12.2, 9.8, 3.7 Hz, CHAr), 2.16-2.09 (m, 1H, CHCHOH), 1.92-1.83 (m, 2H,

CHCHAr and CHCHOH), 1.83-1.72 (m, 1H, CHCHAr), 1.63-1.26 (m, 5H, CH_2CH_2, OH); exact mass 176.1203 (176.1201 calcd for $C_{12}H_{16}O$). Anal. Calcd.for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 82.01; H, 9.10.

For **79**: IR ($CHCl_3$ cast) 2933, 1736, 1372, 1241 cm^{-1} ; ^1H NMR (360 MHz, $CDCl_3$) δ 7.32-7.16 (m, 5H, ArH), 4.97 (ddd, 1H, *J*= 10.4, 10.4, 4.3 Hz, CHOR), 2.65 (ddd, 1H, 11.0, 11.0, 3.7 Hz, CHAr), 2.19-2.10 (m, 1H, CH_aHCHOR), 2.00-1.86 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.78 (s, 3H, CH_3), 1.64-1.30 (m, 4H, CH_2CH_2); exact mass 218.1287 (218.1307 calcd for $C_{14}H_{18}O_2$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*S*, 2*R*)-*trans*-2-Phenylcyclohexanol (80)

The method of Whitesell and Lawrence[327] was used. A solution of **79** (46.3 g, 21.2 mmol) and 85% KOH (30.4 g, 460 mmol) in MeOH (220 mL) was stirred at 60 °C for 1.5 h. The mixture was concentrated *in vacuo* and the residue was partitioned between water (200 mL) and EtOAc (250 mL). The aqueous phase was further extracted with EtOAc (1 x 250 mL, 3 x 100 mL), and the combined organic extracts were dried over Na_2SO_4. Concentration *in vacuo* yielded a yellow solid which upon repeated recrystallization from distilled pentane afforded 16.9 g (45%) of alcohol **80**: mp 64.5-65 °C; $[\alpha]_D^{26.5}$= +58.6 ° (c= 0.85, MeOH) (lit.[327] $[\alpha]_D$= +58.3 ° (c=10, MeOH)); IR ($CHCl_3$ cast) 3290, 2937, 2923, 2920, 1445, 1061, 1051 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.36-7.21 (m, 5H, ArH), 3.66 (ddd, 1H, *J*= 10.4, 10.4, 4.3 Hz, CHOH), 2.43 (ddd, 1H, *J*= 12.2, 9.8, 3.7 Hz, CHAr), 2.16-2.06 (m, 1H, CH_aHCHOH), 1.92-1.70 (m, 3H, CHH_bCHOH, CH_2CHAr), 1.60-1.22 (m, 5H, CH_2CH_2, OH); exact mass 176.1200 ((176.1201 calcd for $C_{12}H_{16}O$). Anal. Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 82.06; H, 8.79.

(1*R*, 2*S*) *trans*-2-Phenylcyclohexyl (2*R*)-2-Methoxy-2-trifluoromethylphenylacetate (83) [336]

The procedure of Kharasch and Brown[364] was followed to prepare the required acid chloride. *R* (+)MTPA (0.193g, 0.800 mmol) was dissolved in 5 ml oxalyl chloride and stirred for 1.5 hours. The oxalyl chloride was removed *in vacuo* and the residue was concentrated *in vacuo* with dry benzene (3 x 5 mL). The acid chloride **81** was dissolved in dry CCl_4 (1.00 mL) and used in the esterifications. For the esterifications the method of Dale and Mosher[365] was modified. Compound **81** in dry CCl_4 (0.800 mM, 0.32 mL) was added to a solution of **75** (0.0304 g, 0.172 mmol) in dry pyridine (0.30 mL) and the solution was stirred at room temperature for 2 hours. The reaction was quenched by the addition of hexanediamine (0.0538 g, 0.463 mmol). The mixture was stirred a further 5 min before ether (5.0 mL) was added. The slurry was washed with cold 1M HCl (5.0 mL), cold saturated Na_2CO_3 (5.0 mL), and cold brine (5.0 mL). The organic portion was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (10% EtOAc/ hexanes, EtOAc) to give 0.0398 g (59%) of a colorless oil; $[\alpha]_D$= +21.0 ° (c= 2.0, $CHCl_3$); IR ($CHCl_3$ cast) 2939, 2860, 1745, 1495, 1450, 1291, 1271, 1248, 1181, 1168, 1120, 1021 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-6.94 (m, 10H, ArH), 5.28 (ddd, 1H, *J*= 10.7, 10.7, 4.4 Hz, CHOR), 3.11 (d, 3H, *J*= 1.2 Hz, OCH_3), 2.80 (ddd, 1H, *J*= 12.2, 10.7, 3.8 Hz, CHAr), 2.27-2.14 (m, 1H, CH_aHCHOR), 2.05-1.19 (m, 7H, CHH_bCHOR, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.9 (CO), 142.9 (Ar), 132.2 (Ar), 129.1 (Ar), 128.7 (Ar), 128.1 (Ar), 127.7 (Ar), 127.0 (Ar), 126.8 (Ar), 123.3 (CF_3), 79.0 (CHOR), 54.9 (CHAr), 49.4 (OCH_3), 34.4 (CH_2CHOR), 31.7 (CH_2CHAr), 26.0 (CH_2), 25.0 (CH_2); exact mass 392.1604 (392.1599 calcd for $C_{22}H_{23}O_3F_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*S*, 2*R*) *trans*-2-Phenylcyclohexyl (2*R*)-2-Methoxy-2-trifluoromethylphenylacetate (84)

Compound **84** was prepared from **80** in 78% yield by the same method used for the conversion of **75** to **83**, employing 22.3 mg (0.126 mmoL) of **80**, 0.23 mL (0.825 M) MTPACl (**81**) in CCl_4, and quenching with 22.4 mg (0.192 mmol) hexanediamine:$[\alpha]_D$= +18.7 ° (c= 0.70, $CHCl_3$); IR ($CHCl_3$ cast) 2937, 1744, 1495, 1450, 1290, 1180, 1169, 1120, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33-7.03 (m, 10H, ArH), 5.35 (ddd, 1H, *J*=10.7, 10.7, 4.4 Hz, CHOR), 3.21 (d, 3H, *J*= 1.2 Hz, OCH_3), 2.73 (ddd, 1H, *J*= 11.7, 10.7, 3.6, CHAr) 2.31-2.18 (m, 1H, CH_aHCHOR), 2.02-1.23 (m, 7H, CHH_bCHOR, $CH_2CH_2CH_2$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 165.7 (CO), 142.4 (Ar), 132.2 (Ar), 129.0 (Ar), 128.4 (Ar), 128.1 (Ar), 127.6 (Ar), 127.1 (Ar), 126.6 (Ar), 122,3 (CF_3), 78.5 (CHOR), 55.0 (CHAr), 49.5 (OCH_3), 34.4 (CH_2CHOR), 32.1 (CH_2CHAr), 25.6 (CH_2), 24.6 (CH_2); exact mass 392.1586 (392.1599 calcd for $C_{22}H_{23}O_3F_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*RS*, 2*SR*) *trans*-2-Phenylcyclohexyl (2*R*)-2-Methoxy-2-trifluoromethylphenylacetate (82)

Compound **82** was prepared from **74** in 16% yield by the same method used to prepare **83** from **75**, using 36.0 mg (0.204 mmol) **74** and 0.27 mL (2.25 M) MTPACl (**81**) in CCl_4, and quenching with 39.7 mg (0.342 mmol) hexanediamine: IR ($CHCl_3$ cast) 2938, 2859, 1744, 1495, 1463, 1271, 1181, 1168, 1021 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46-6.95 (m, 10H, ArH), 5.40-5.22 (overlapping ddd, 1H, CHOR), 3.20 (d, 0.75H, *J*= 1.1 Hz, OCH_3), 3.11 (d, 2.25H, *J*= 1.2 Hz, OCH_3), 2.82-2.67 (overlapping ddd, 1H, CHAr) 2.26-2.14 (m, 1H, CH_aHCHOR), 2.02-1.23 (m, 7H, CHH_bCHOR, $CH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0 (CO), 165.7 (CO), 142.9 (Ar), 132.5 (Ar), 129.1 (Ar), 128.7 (Ar), 128.4 (Ar), 128.2 (Ar), 127.7 (Ar), 127.1 (Ar), 126.8 (Ar),126.6 (Ar), 123.2 (CF_3), 79.0 (CHOR) 78.5 (CHOR), 77.2 (CR_4), 55.0

(CHAr), 49.5 (OCH_3), 34.44 (CH_2CHOR), 34.41 (CH_2CHOR), 32.2, (CH_2CHAr), 31.7 (CH_2CHAr), 25.6 (CH_2), 24.6 (CH_2); exact mass 392.1603 (392.1599 calcd for $C_{22}H_{23}O_3F_3$). Anal Calcd for $C_{22}H_{23}O_3F_3$: C, 67.34; H, 5.91. Found: C, 66.96; H, 5.84.

(1*R*, 2*S*)-*trans* -2-Phenylcyclohexyl Bromoacetate (85) [251]

The procedure of Whitesell and coworkers[251] was followed. *p*-Toluenesulfonic acid (0.11 g, 0.64 mmol) was added to a solution of **75** (4.00 g, 22.7 mmol) and bromoacetic acid (8.00 g, 57.6 mmol) in benzene (55.0 mL), and the solution was heated to reflux with azeotropic removal of water for 18 h. The mixture was cooled and poured into saturated $NaHCO_3$ (100 mL). The organic layer was separated and the aqueous layer was extracted with ether (3 x 50 mL). The combined organic layers were washed with brine (2 x 25 mL), dried over Na_2SO_4, then concentrated *in vacuo* to give 6.75 g (quantitative yield) of an oil:$[\alpha]_D$= -21.0 ° (c= 1.1, $CHCl_3$); IR ($CHCl_3$ cast) 2935, 1731, 1278cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.31-7.12 (m, 5H, ArH), 5.02 (ddd, *J*= 11.0, 11.0, 4.3 Hz, 1H, CHO), 3.54 (d, *J*=12.2 Hz, 1H, CHBr), 3.48 (d, *J*=12.2 Hz, 1H, CHBr), 2.69 (ddd, *J*=12.2, 11.0, 3.7 Hz, 1H, CHAr), 2.22-2.08 (m, 1H, CHCHO), 2.00-1.75 (m, 3H, CHCHO, CH_2Ar), 1.65-1.23 (m, 4H, CH_2CH_2);^{13}C NMR (90.5 MHz, $CDCl_3$) δ 166.4 (CO_2R), 142.5 (Ar),128.3 (Ar), 127.5 (Ar), 127.5 (Ar), 126.6 (Ar), 78.1 (CHOR), 49.6 (CHAr), 33.7 (CH_2), 31.9 (CH_2), 25.9 (CH_2Br), 25.7 (CH_2), 24.6 (CH_2); exact mass 296.0414 (296.0412 calcd for $C_{14}H_{17}O_2Br$). Anal. Calcd for $C_{14}H_{17}O_2Br$: C, 56.58; H, 5.76; Br, 26.89. Found: C, 56.78; H, 5.62; Br, 26.90.

(1*S*, 2*R*)-*trans* -2-Phenylcyclohexyl Bromoacetate (86)

Bromoacetate **86** was prepared from **80** (5.00 g, 28.4 mmol) in 99% yield by the same procedure as for **85**, using 10.1 g (72.5 mmol) bromoacetic acid and 0.11g (0.64 mmol)

p-TsOH: $[\alpha]_D$= +19.5 ° (c=3.2, $CHCl_3$); spectral properties of **86** were the same as for **85**.

(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl Nitrooxyacetate (87)

The procedure of Whitesell and coworkers[251] was used. $AgNO_3$ (9.70 g, 57.1 mmol) was added to a solution of **85** (6.25 g, 21.0 mmol) in CH_3CN (52 mL). The mixture was stirred at room temperature for 23 h, and additional $AgNO_3$ (1.03 g, 6.06 mmol) was added and the mixture was stirred a further 21 h. Concentration *in vacuo* gave a green residue which was extracted with ether (75 mL). The AgBr solid was filtered and washed thoroughly with ether. The combined ether extracts were washed with water (2 x 50 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give 5.79 g (99% yield) of a white solid: mp 74-75 °C; $[\alpha]_D$= +14.5 ° (c= 0.30, $CHCl_3$); IR ($CHCl_3$ cast) 2947, 1750, 1669, 1292, 1230 cm^{-1}; ^1H NMR 7.32-7.12 (m, 5H, ArH), 5.09 (ddd, 1H, *J*=11.0, 10.4, 4.3 Hz, CHOR), 4.65 (d, 1H, *J*=17.1 Hz, CH_aHONO_2), 4.49 (d, 1H, *J*=17.1 Hz, CHH_bONO_2), 2.68 (ddd, 1H, *J*= 12.2, 11.0, 3.7, CHAr), 2.23-2.10 (m, 1H, CHCHOR), 2.02-1.76 (m, 3H, CHCHOR, CH_2CHAr), 1.66-1.28 (m, 4H, CH_2CH_2);^{13}C NMR (90.5 MHz, $CDCl_3$) 165.0 (CO_2R),142.2 (Ar), 128.4 (Ar), 127.3 (Ar), 126.7 (Ar), 78.3 (CHOR), 66.9 (CH_2NO_2), 49.6 (CHAr), 33.6 (CH_2), 32.0 (CH_2), 25.6 (CH_2), 24.6 (CH_2); exact mass 279.1083 (279.1107 calcd for $C_{14}H_{17}NO_5$). Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.21; H, 6.14; N, 5.01. Found: C, 60.14; H,6.16; N,5.18.

(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl Nitrooxyacetate (88)

Nitrooxyacetate **88** was prepared in 98% yield from **86** (7.80 g, 26.2 mmol) in the same manner as **87** from **85**, employing 11.2 g (65.9 mmol) $AgNO_3$ and 55 mL CH_3CN: mp 74.5-75 °C; $[\alpha]_D$= -13.3 °; remaining spectral properties are the same as for **87**.

(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl Oxoacetate (89)

The procedure of Whitesell and coworkers[251] was used. Compound **87** (5.57 g, 19.9 mmol) and sodium acetate trihydrate (2.74 g, 20.1 mmol) were dissolved in DMSO (95.0 mL) and stirred for 2 h at room temperature. The solution was poured into ice/salt water (100 mL), extracted with ether (5 x 50 mL), and the combined ether extracts were washed with brine (2 x 50 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give 4.68 g (94% yield) of a pale yellow solid: mp 62.5 -64.5 °C; IR ($CHCl_3$ cast) 3480 br, 2935, 2859, 1746, 1285, 1253, 1223 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.16 (s, 0.25 H,CHO), 7.36-7.12 (m, 5H, ArH), 5.21-4.85 (m, 1.75H, CHOR, $CH(OH)_2$), 2.90-2.63 (m, 1H, CHAr), 2.24-1.27 (m, 10H, $CH(OH)_2$, 4 CH_2); ^{13}C NMR (90.5 MHz, $CDCl_3$) 183.9 (CHO), 169.7 (CO_2R), 142.3 (Ar), 128.4 (Ar), 128.3 (Ar), 127.4 (Ar), 86.8 ($CH(OH)_2$), 78.3 (CHOR), 49.5 (CHAr), 33.6 (CH_2), 31.9 (CH_2), 25.6 (CH_2), 24.6 (CH_2); exact mass 232.1099 (232.1099 calculated for $C_{14}H_{16}O_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl Oxoacetate (90)

The method of Whitesell *et al.*[251] was modified. Sodium acetate trihydrate (3.25 g, 23.9 mmol) and **88** (6.65 g, 23.8 mmol) were dissolved in DMSO (100 mL) and the solution was stirred at room temperature for 1 h. The mixture was poured into ice/ saturated $NaHCO_3$ (100 mL) and the aqueous solution extracted with ether (3 x 50 mL). The aqueous phase was then acidified with AcOH and was extracted with ether (3 x 50 mL). The combined ether layers were washed with brine (2 x 50 mL) and were dried over Na_2SO_4. Concentration *in vacuo* afforded 5.48 g (92%) of a faintly yellow solid. A small portion was heated to 68-74 °C at 0.5 mm Hg to give a colorless gum: mp (hydrate) 78.5-79.5 °C; IR ($CHCl_3$ cast) 3470, 2935, 1743, 1224 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.16 (s, 0.25 H,CHO), 7.36-7.12 (m, 5H, ArH), 5.21-4.85 (m, 1.75H, CHOR, $CH(OH)_2$), 2.90-2.63 (m, 1H, CHAr), 2.24-1.27 (m, 10H, $CH(OH)_2$, 4 CH_2); ^{13}C

NMR (90.5 MHz, $CDCl_3$) 183.9 (CHO), 169.7(CO_2R), 142.3 (Ar), 128.4 (Ar), 128.3 (Ar), 127.5 (Ar), 86.8 ($CH(OH)_2$), 78.3(CHOR), 49.5 (CHAr), 33.6 (CH_2), 31.9 (CH_2), 25.6 (CH_2), 24.6 (CH_2); exact mass 232.1097 (232.1099 calcd for $C_{14}H_{16}O_3$). Anal. Calcd for $C_{14}H_{16}O_3$; C, 67.18; H, 7.25. Found: C, 67.46; H, 7.36.

1-Methyl 7-[(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-Carbobenzyloxy)amino-6-hydroxy-3-heptenedioate (91)

The combined and modified procedures of Girodeau *et al.*[60] and Whitesell *et al*[252] were used. $SnCl_4$ (10.0 mL, 86.7 mmol) was added over 2 min to a stirred solution of **89** (5.01 g, 20.0 mmol) in dry CH_2Cl_2 (125 mL) at approx -55 °C. The solution was cooled to -78 °C and stirred for 5 min, and **68** (5.86 g, 22.3 mmol) in dry CH_2Cl_2 (40 mL) was added over 15 min. The solution was warmed to -23 °C and was stirred for 3 h. The mixture was quenched with ether (500 mL) and washed with saturated $NaHCO_3$ (2 x 250 mL) and water (250 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give a pale yellow oil. Purification by flash chromatography (EtOAc 40% / hexane 60%) gave 8.20 g (83%) of a clear oil:$[\alpha]_D$= +28.1 ° (c=1.9, $CHCl_3$); IR ($CHCl_3$ cast) 3420, 3360, 2936, 1726, 1271, 1208 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.44-7.10 (m, 10H, ArH), 5.55 (d, 1H, *J*=7.9 Hz, NH), 5.25-4.92 (m, 5H, CHOR, CH=CH, CHAr), 4.75-4.64 (m, 1H, CHNH), 3.96 (dd, 1H, J= 11.0, 6.0, Hz, CHOH), 3.68 (s, 3H, OCH_3) 2.90 (br s, 1H, OH), 2.65 (m, 1H, CHAr), 2.18-2.00 (m, 1H, CH_aHCHOR) 2.00-1.18 (m, 9H, CH_2CH, CHH_bCHOR, CH_2CHAr, CH_2CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.3 (CO_2R), 171.0 (CO_2R), 155.4 (CONH), 142.8 (Ar), 136.2 (CH=), 128.5 (Ar), 128.3 (Ar), 127.55(CH=), 127.5(Ar), 126.8 (Ar), 78.0 (CHOR), 69.3 (CHOH), 67.0 (CH_2Ar), 55.3 (CHNH), 52.5 (OCH_3), 49.8 (CHAr), 36.5 (CH_2), 33.7 (CH_2), 32.1 (CH_2), 25.6 (CH_2), 24.6 (CH_2); exact mass 495.2259 (495.2257 calcd for $C_{28}H_{33}NO_7$). Anal. Calcd for $C_{28}H_{33}NO_7$: C, 67.86; H, 6.71; N, 2.83. Found: C, 67.55; H, 6.88; N, 2.90.

1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-Carbobenzyloxy)amino-6-hydroxy-3-heptenedioate (92)

Alcohol **92** was prepared in 81% yield from the condensation of **90** (1.05 g, 4.20 mmol) and **68** (1.01 g, 3.84 mmol) in the same manner as for **91**, using 1.75 mL $SnCl_4$ (15.2 mmoL) and 20 mL CH_2Cl_2: $[\alpha]_D$= +24.4 ° (c=0.85, $CHCl_3$); IR ($CHCl_3$ cast) 3420, 3360, 2936, 1726, 1265, 1209 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.34-7.06 (m, 10H, ArH), 5.54 (d, 1H, *J*=8.3 Hz, NH), 5.25-4.90 (m, 5H, CHOR, CH=CH, CHAr), 4.72-4.64 (m, 1H, CHNH), 4.04-3.96 (m, 1H, CHOH), 3.68 (s, 3H, OCH_3) 2.90 (d, 1H, *J*=6.0 Hz, OH), 2.65 (ddd, 1H, *J*= 12.2, 11.0, 3.7 Hz, CHAr), 2.18-2.00 (m, 1H, CH_aHCHOR) 2.05-1.22 (m, 9H, CH_2CH, CHH_bCHOR, CH_2CHAr, CH_2CH_2); ^{13}C-NMR (90.5 MHz, $CDCl_3$) δ 173.4 (CO_2R), 171.1 (CO_2R), 155.4 (CONH), 142.8 (Ar), 136.2 (CH=), 128.5 (Ar), 128.3 (Ar), 127.8(CH=), 127.52(Ar) 127.48 (Ar), 127.4 (Ar), 127.3 (Ar), 126.8 (Ar), 78.1 (CHOR), 69.4 (CHOH), 67.0 (CH_2Ar), 55.4 (CHNH), 52.5 (OCH_3), 49.8 (CHAr), 36.5 (CH_2), 33.8 (CH_2), 32.2 (CH_2), 25.6 (CH_2), 24.6 (CH_2); exact mass 495.2256 (495.2257 calcd for $C_{28}H_{33}NO_7$). Anal. Calcd for $C_{28}H_{33}NO_7$: C, 67.86; H, 6.71; N, 2.83. Found: C, 68.02; H, 6.93; N, 2.96. Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl (1*S*, 2*R*) *trans*-2-Phenylcyclohexyl (2*S*,6*S*)-2-(*N*-Carbobenzyloxy)amino-6-[(2*R*)-2-methoxy-2-trifluoromethylphenylacetoxy]-3-heptenedioate (94)

The procedures of Davisson et al [366] and Boden and Keck[338] were modified. DCC (87.4 mg 0.424 mmol) was added to a solution of DMAP (51.5 mg, 0.422 mmol), and DMAP·HCl (48.3 mg, 0.304 mmol) and MTPA (56.7 mg, 0.242 mmol) in dry CH_2Cl_2 (2 mL), and the solution was stirred at room temperature for 1h. Alcohol **91** (43.7 mg, 0.0881 mmol) in dry CH_2Cl_2 (2 mL) was added and the mixture was stirred for 22 h .

The precipitate was filtered, and the filtrate was diluted with 15 mL of CH_2Cl_2 and was washed with 1M HCl (2 x 10 mL) and saturated $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was suspended in EtOAc and hexanes and filtered to remove additional *N,N'*-dicyclohexylurea. The filtrate was concentrated *in vacuo* to give an oil, a portion (28.3 mg) of which was purified by flash chromatography (10% EtOAc/ 90% hexanes, 30% EtOAc/ 70% hexanes) to give 15.1 mg (53%) of a colorless oil: IR ($CHCl_3$ cast) 3490, 2929, 1748, 1729, 1262, 1205, 1190,1057, 1045 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.57-7.02 (m, 15H, ArH), 5.38-4.93 (m, 6H, NH, CHOR, CH=CH, CH_2Ar), 4.90 (dd, 1H, *J*=6.9, 4.7 Hz, CHO_2CR_3) 4.76-4.68 (m, 1H, CHNH), 3.69 (s, 3H, CO_2CH_3) 3.45 (s, 3H, OCH_3) 2.72-2.62 (m, 1H, CHAr), 2.27-1.27 (m, 10H, $CH_2CHO_2CR_3$, CH_2CHOR, CH_2CHAr, CH_2CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.8 (C=O), 167.3 (C=O), 166.0 (C=O),142.8 (Ar), 136.2 (CH=), 131.6 (Ar), 129.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.34 (Ar), 128.25 (Ar), 128.2 (Ar), 127.8 (CH=), 127.5(Ar), 127.2 (Ar), 126.8 (Ar), 123.0 (CF_3), 78.1 (CHOR),77.2 (CR_4), 73.1 (CHO_2CR_3), 67.1 (CH_2Ar), 55.4 (OCH_3), 55.3 (CHNH), 52.6 (OCH_3), 49.9 (CHAr), 33.9 (CH_2), 32.9 (CH_2), 32,85 (CH_2),32.2 (CH_2), 25.7 (CH_2), 24.7 (CH_2); MS (NH_3 CI) 729 (729 calcd for M + NH_4^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl (1*R*, 2*S*) *trans*-2-Phenylcyclohexyl (2*S*,6*R*)-2-(*N*-Carbobenzyloxy)amino-6-[(2*R*)-2-methoxy-2-trifluoromethylphenylacetoxy]-3-heptenedioate (93)

The same procedure used to convert **91** to **94** was used to prepare **93** from **92** (13.1 mg, 0.0263 mmol) in 37% yield, using 24.7 mg (0.105 mmol) MTPA, 13.6 mg (0.111 mmol) DMAP, 9.84 mg (0.0620 mmol) DMAP·HCl, and 44.0 mg (0.213 mmol) DCC: IR ($CHCl_3$ cast) 3400, 2940, 1748, 1733, 1271, 1208, 1190, 1171,1057, 1047 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.63-7.07 (m, 15H, ArH), 5.20-4.92 (m, 6H, NH, CHOR,

CH=CH, CH_2Ar), 4.90 (dd, 1H, *J*=11, 5.1 Hz, CHO_2CR_3) 4.68-4.59 (m, 1H, CHNH), 3.68 (s, 3H, CO_2CH_3) 3.54 (s, 3H, OCH_3) 2.72-2.62 (m, 1H, CHAr), 2.27-1.27 (m, 10H, $CH_2CHO_2CR_3$, CH_2CHOR, CH_2CHAr, CH_2CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.7 (C=O), 167.5 (C=O), 166.0 (C=O), 155.5 (C=O), 142.8 (Ar), 136.2 (CH=), 131.9 (Ar) 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.18 (Ar), 128.15 (Ar), 127.54 (CH=), 127.49 (Ar), 1270 (Ar), 126.8 (Ar), 123.1 (CF_3), 78.1 (CHOR), 77.2 (CR_4), 73.1 (CHO_2CR_3), 67.1 (CH_2Ar), 55.5(OCH_3), 55.1 (CHNH), 52.5 (OCH_3), 49.8 (CHAr), 33.9 (CH_2), 32.9 (CH_2), 32.2 (CH_2), 25.6 (CH_2), 24.6 (CH_2);MS (NH_3 CI) 729 (729 calcd for M + NH_4^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl (1*RS*, 2*SR*) *trans*-2-Phenylcyclohexyl (2*S*,6*RS*)-2-(*N*-Carbobenzyloxy)amino-6-[(2*R*)-2-methoxy-2-trifluoromethylphenylacetoxy]-3-heptenedioate (95)

Compound **95** was prepared from a 2:1 mixture of **91** and **92** in 55% yield by the same method used to prepare **94** from **91**, using 24.6 mg (0.0497 mmol) of the alcohol mixture, 26.9 mg (0.115 mmol) of MTPA, 40.3 mg (0.330 mmol) DMAP, 56.2 mg (0.354 mmol) DMAP·HCl, and 53.7 mg (0.260 mmol) DCC; IR ($CHCl_3$ cast) 2937, 1748, 1733, 1270, 1192, 1172, 1058 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.62-7.02 (m, 15H, ArH), 5.40-4.90 (m, 6H, NH, CHOR, CH=CH, CH_2Ar), 4.90 (overlapping dd, 1H, CHO_2CR_3), 4.76-4.60(overlapping m, 1H, CHNH), 3.69 (s, 3H, CO_2CH_3), 3.57 (d, 0.33H, *J*= 0.9 Hz, OCH_3), 3.54 (d, 0.84H, *J*=0.9Hz, OCH_3), 3.45 (s, 1.83H, OCH_3) 2.72-2.62 (m, 1H, CHAr), 2.27-1.27 (m, 10H, $CH_2CHO_2CR_3$, CH_2CHOR, CH_2CHAr, CH_2CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.8 (C=O), 167.6 (C=O), 167.2 (C=O), 166.0 (C=O), 165.97 (C=O), 155.4 (C=O)142.8 (Ar), 136.2 (CH=), 131.9 (Ar), 131.6 (Ar), 129.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.34 (Ar), 128.24 (Ar), 128.2 (Ar), 128.16 (Ar) 127.8 (CH=), 127.55 (Ar), 127.49 (Ar), 127.1

(Ar), 126.82 (Ar), 126.78 (Ar), 123.0 (CF_3), 78.1 (CHOR), 78.0 (CHOR), 77.2 (CR_4), 73.1 (CHO_2CR_3), 73.0 (CHO_2CR_3), 67.1 (CH_2Ar), 55.5 (OCH_3), 55.4 (OCH_3), 55.2 (CHNH), 55.1 (CHNH), 52.6 (OCH_3) 52.5 (OCH_3), 49.86 (CHAr),49.81 (CHAr), 36.5 (CH_2), 33.9 (CH_2), 32.9 (CH_2), 32,85 (CH_2), 32.8(CH_2),32.2 (CH_2), 32.16(CH_2), 25.7 (CH_2), 24.7 (CH_2); MS (CI NH_3) 729 (729 calcd for M+ NH_4^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

1-Methyl 7-[(1*R*, 2*S*)-trans-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-carbobenzyloxy)amino-6-[(methylsulfonyl)oxy]-3-heptenedioate (97)

The method of Girodeau[60] was adapted. Methanesulfonyl chloride (98% , 0.31 mL, 3.93 mmol) was added to a solution of **92** (0.960 g, 1.938 mmol) in dry pyridine (20 mL), and the mixture stirred for 1.25 h. The reaction was quenched by the addition of 20% $CuSO_4 \cdot 5 H_2O$ (20 mL), and then the solution was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with 2M HCl (3 x 25 mL) and dried over Na_2SO_4. Concentration *in vacuo* gave 1.26 g (92%) of an orange amorphous solid :$[\alpha]_D$= +22.8 ^{o} (c=0.32, $CHCl_3$); IR ($CHCl_3$ cast) 3370, 2937, 1749, 1726, 1362, 1271, 1213, 1176, 1029 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.44-7.05 (m, 10H, ArH), 5.48-5.24 (m, 2H, CH=CH), 5.12 (s, 2H, CH_2), 5.08-4.96 (m, 1H, CHOR,), 4.82-4.72 (m, 2H, CHNH, $CHOSO_2$), 3.76 (s, 3H, OCH_3), 2.92 (s, 3H, OSO_2CH_3), 2.75-2.64 (m, 1H, CHAr), 2.22-1.70 (m, 6H, 3 CH_2) 1.62-1.26 (m, 4H, 2 CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.6 (CO_2R), 167.3 (CO_2R), 155.3 (CONH), 142.5 (Ar), 136.0 (CH=), 128.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (CH=), 127.9 (Ar), 127.3 (Ar), 126.7 (Ar), 78.2 (CHOR), 76.4($CHOSO_2$), 66.9 (CH_2Ar), 55.2 (CHNH), 52.5 (OCH_3), 49.6 (CHAr), 38.6 (CH_2), 34.0 (CH_2), 33.9 (CH_3), 31.9 (CH_2), 25.4 (CH_2), 24.4 (CH_2); exact mass • 573.2011 (573.2032 calcd for $C_{29}H_{35}NO_9S$). Anal. Calcd for $C_{29}H_{35}NO_9S$: C, 60.72; H, 6.15; N, 2.44; S, 5.59. Found: C, 60.99; H, 6.34; N, 2.56; S, 5.31.

1-Methyl 7-[(1*S*, 2*R*)-trans-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-Carbobenzyloxy)amino-6-[(methylsulfonyl)oxy]-3-heptenedioate (96)

The procedure used to convert **92** to **97** was used to prepare **96** from **91** (0.800 g, 1.62 mmol) in 96% yield, using methanesulfonyl chloride (0.260 mL, 3.29 mmol) in pyridine (20 mL): $[\alpha]_D$= +23.3 ° (c=3.3, $CHCl_3$); IR ($CHCl_3$ cast) 3370, 2938, 1749, 1726, 1363, 1263, 1209, 1176, 1045, 1029 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.42-7.10 (m, 10H, ArH), 5.43-5.24 (m, 2H, CH=CH), 5.12 (s, 2H, CH_2), 5.09-4.98 (m, 1H, CHOR), 4.86-4.72 (m, 2H, CHNH, $CHOSO_2$), 3.75 (s, 3H, OCH_3), 2.88 (s, 3H, OSO_2CH_3), 2.74-2.62 (m, 1H, CHAr), 2.20-1.23 (m, 10H, 5 CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.6 (CO_2R), 167.3 (CO_2R), 155.3 (CONH), 142.5 (Ar), 136.1 (CH=), 128.9 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (CH=), 127.9 (Ar), 127.3 (Ar), 126.7 (Ar), 126.3 (Ar), 78.2 (CHOR), 76.3 ($CHOSO_2$), 66.8 (CH_2Ar), 55.1 (CHNH), 52.5 (OCH_3), 49.5 (CHAr), 38.5 (CH_2), 34.0 (CH_2), 33.8 (CH_3), 31.9 (CH_2), 25.4 (CH_2), 24.4 (CH_2); exact mass 573.1997 (573.2032 calcd for $C_{29}H_{35}NO_9S$). Anal. Calcd for $C_{29}H_{35}NO_9S$: C, 60.72; H, 6.15; N, 2.44; S, 5.59. Found: C, 60.41; H, 6.31; N, 2.47; S, 5.29.

1-Methyl 7-[(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-Carbobenzyloxy)amino-6-azido-3-heptenedioate (98)

The method of Girodeau[60] was adapted. NaN_3 (0.54 g, 8.3 mmol) followed by dry DMF (4 mL) was added to a solution of **96** in dry DMF (10 mL). The mixture was stirred at room temperature for 21 h. The solvent was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 (75 mL). The solution was washed with brine (2 x 25 mL) and the organic phase was dried over Na_2SO_4. Evaporation of the solvent gave 0.86 g (quantitative yield) of an unstable orange-brown oil: IR($CHCl_3$ cast) 3330, 2937, 2107, 1732, 1452, 1269, 1207 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.45-7.02 (m, 10H, ArH), 6.30 (br s, 1H, NH), 5.58-5.26 (m, 2H, CH=CH), 5.22-4.95 (m, 3H, CHOR, CH_2Ar),

4.88-4.74 (m, 1H, CHNH), 3.76 (s, 3H, OCH_3),3.58-3.40 (m, 1H, CHN_3), 2.80-2.66 (m, 1H, CHAr), 2.28-1.16 (m, 10H, 5 CH_2); MS (Pos FAB, Cleland) 521.29 (521.23 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-Carbobenzyloxy)amino-6-azido-3-heptenedioate (99)

The same procedure as used for the conversion of **96** to **98** was used for the synthesis of **99** from **97** (0.750 g, 1.31 mmol) in quantitative yield, using sodim azide (0.430 g, 6.61 mmol) in DMF (10 mL): IR($CHCl_3$ cast) 3360, 2938, 2106, 1737, 1265, 1207 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.42-7.02 (m, 10H, ArH), 5.52-5.29 (m, 2H, CH=CH), 5.18-4.94 (m, 1H, CHOR), 5.02 (s, 2H, CH_2), 4.84-4.74 (m, 1H, CHNH), 3.72 (s, 3H, OCH_3),3.52-3.40 (m, 1H, CHN_3), 2.76-2.62 (m, 1H, CHAr), 2.26-1.22 (m, 10H, 5 CH_2); exact mass 492.2260 (492.2260 calcd for $C_{28}H_{32}N_4O_6$-N_2), MS (Pos FAB, glycerol) 521.28 (521.23 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

(2*S*, 6*R*)-2-(*N*-Carbobenzyloxy)amino-6-azidoheptanedicarboxylic Acid (101)

The procedure of Evans *et al.* [367] was used. $LiOH{\cdot}H_2O$ (9.77 mg, 0.233 mmol) was added to a cooled (0 °C) solution of **99** (19.7 mg, 0.0379 mmol) in THF (3 mL) and water (1 mL). The mixture was stirred 1 hour and saturated $NaHCO_3$ (1mL) was added . The THF was removed *in vacuo* and additional saturated $NaHCO_3$ (1mL) and water (3 mL) were added. The solution was extracted with CH_2Cl_2 (3 x 5 mL) and the aqueous layer was acidified to pH 1 with 5.7 M HCl. The aqueous phase was extracted with EtOAc (3 x 5 mL) and the extracts were dried over Na_2SO_4. Concentration *in vacuo* yielded 11.2 mg (85%) of an oil: IR ($CHCl_3$ cast) 3300, 3050, 2963, 2110, 1721, 1261 cm^{-1}; ^1H NMR

(360 MHz, $CDCl_3$)7.40-6.90 (br s, 2H, 2 CO_2H), 7.36 (s, 5H, ArH), 5.96-5.68 (m, 2H, CH=CH), 5.16 (s, 2H, CH_2Ar), 5.04- 4.80 (m, 1H, CHNH), 4.22-4.00 (m, 1H, CHN_3), 2.64-2.58 (m, 2H, CH_2CHN_3); MS (Pos FAB Cleland) found 370.92 (371.10 calc for $M+Na^+$) and 348.76 (348.32 calcd for $C_{15}H_{16}N_4O_6$). Purity of the material was determined to be greater than 85% by ^1H NMR.

(2*S*,6*S*)-2-(*N*-Carbobenzyloxy)amino-6-azidoheptanedicarboxylic Acid (100)

The same procedure was that used to produce **101** from **99** was used to prepare **100** from **98** in 85% yield: spectral properties of **100** are identical to those for **101**.

1-Methyl 7-(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl 2,6-diaminoheptanedioate (102) Procedure 1

Pd on carbon (5%, 0.0180 g) was added to a solution of **98** (0.0509 g, 0.0977 mmol) in AcOH (5 mL), and the mixture was shaken under H_2 (52 psi) for 3 d. The mixture was filtered through Celite, and the Celite was washed with AcOH. The filtrate was concentrated *in vacuo* to give 0.0567 g (quant.) of an oil: IR (CH_3OH cast) 3420, 1731, cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.37-6.75 (m, 5H, ArH), 5.08-4.78 (m, 1H, CHOR), 3.78-3.50 (m, 2H, CHNH), 3.00-0.75 (m, 20H, 7 CH_2, 2 $OCOCH_3$); MS (Pos FAB, Cleland) found 363.19 (363.22 calcd for MH^+). Purity of the material was determined to be greater than 80% by ^1H NMR.

1-Methyl 7-(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl 2,6-Diaminoheptanedioate (103) Procedure 1

Azide **99** (0.409 g, 0.786 mmol) was dissolved in AcOH (10 mL) and added to 5% Pd on carbon (0.986 g) in a Parr bottle. The apparatus was flushed with hydrogen and was then shaken under 48 psi H_2 for 28 h. The mixture was filtered through Celite, and the Celite

was washed with AcOH. The filtrate was lyophilized to give 0.397 g (quant.) of a brown oil which was placed on an ion exchange column and eluted with MeOH and 5% NH_4OH / 95% MeOH to give 0.0793 g (28%) of an oil : IR (CH_3OH cast) 3360, 2934, 1726, 1519, 1261, 1241, 1214 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.42-7.10 (m, 5H, ArH); 4.32-4.15 (m, 1H, CHOR), 3.80 (s, 3H, OCH_3), 3.74-3.68 (m, 1H, CHNH), 3.58-3.40 (m, 1H, CHNH), 2.81-2.70 (m, 1H, CHAr), 2.20-0.90 (m, 14H,7 CH_2); exact mass 362.2210 (362.2205 calcd for $C_{20}H_{30}N_2O_4$). Purity of the material was determined to be greater than 80% by ^1H NMR.

1-Methyl 7-(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl 2,6-Diamino-heptanedicarboxylate (102) Procedure 2

AcOH (0.23 mL) followed by 5% Pd on carbon (0.130 g) was added to a solution of **98** (0.410 gm 0.788 mmol) in MeOH (10 mL). The apparatus was flushed with H_2, and the mixture was stirred under H_2 (1 atm) for 25 h. The mixture was filtered through Celite, and the Celite was washed with MeOH. The filtrate was concentrated *in vacuo* to give 0.356 g (71%) of a yellow oil. Ion exchange chromatography (AGWx8, H^+ form, 90 mL, MeOH, 5% NH_4OH / MeOH) gave 0.0832 g (29%) of an oil: IR (CH_3OH cast), 3260, 2934, 1734, 1689, 1205, 1180 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.38-7.12 (m 5H, ArH), 5.20-5.04 (m 1H, CHOR), 3.81 (s, 3H, OCH_3), 3.55-3.44 (m, 2H, CHNH), 2.84-2.71 (m, CHAr), 2.20-1.06 (m, 14H, 7 CH_2); exact mass 362.2204 (362. 2205 calcd for $C_{20}H_{30}N_2O_4$). Purity of the material was determined to be greater than 85% by ^1H NMR.

1-Methyl 7-(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl 2,6-Diaminoheptanedioate (103) Procedure 2

Following the procedure above, a yield of 18.2 mg (49%) of **103** was obtained usingAcOH (0.030 mL), 5% Pd on carbon (0.0180 g), and **99** (0.0401 g, 0.0769 mmol) in MeOH (1mL). Spectral properties were identical to those of **103** obtained above.

(2*RS*, 6*RS*)- 2,6-Diaminoheptanedioic Acid (1) From 102

The procedure of Evans and Britton was used.[367] $LiOH{\cdot}H_2O$ (4.91 mg, 0.117 mmol) was added to a solution of **102** (8.15 mg, 0.0225 mmol) in 3:1 THF: H_2O (4 mL) and the solution was stirred for 22 h. The THF was removed *in vacuo* and the residue was diluted to 10 mL with H_2O and washed with CH_2Cl_2 (3 x 5 mL). The aqueous layer was concentrated *in vacuo* and the residue was purified by ion exchange chromatography (AG50W x 8, H^+ form , 5 mL) using H_2O and 2% to 10% aqueous NH_4OH as eluents, to give 3.51mg (82%) of a powder: IR (KBr disk) 3440, 2922, 1631cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.74-3,66 (m, 2H, CHNH), 2.05-1,73 (m, 4H, 2 CH_2CHNH), 1.65-1.36 (m, 2H, CH_2); MS (Pos FAB Cleland) found 191.11 (191.09 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

(2*RS*, 6*RS*)- 2,6-Diaminoheptanedioic Acid (1) From 103

The procedure used to prepare **1** from **102** was used to produce **1** from **103** (47.1 mg, 0.0976 mmol) in 46% yield using $LiOH{\cdot}H_2O$ (27.5 mg, 0.656 mmol) and a reaction time of 4 h. Spectral properties were the same as for **1** above.

(2*S*, 6*R*)- 2,6-Diaminoheptanedioic Acid (1a) From 118

Amine **118** (43.9 mg, 0.0948 mmol) was dissolved in MeOH (5 drops) and 5.7 M HCl (6 mL), and heated to reflux for 2.7 h. The solution was cooled and lyophilized, and the residue was purified by ion exchange chromatography (AG50W x 8, H^+ form , 5 mL) to

give 13.1 mg (73%) of a colorless foam. Spectral data were the same as for **1**. Purity of the material was determined to be greater than 90% by ^{1}H NMR.

(2*S*, 6*S*)- 2,6-Diaminoheptanedioic Acid (1b) From 117

Amine **117** (24.0 mg, 0.0517 mmol) was suspended in 5.7 M HCl (5 mL) and heated to reflux for 1.75 h. The solution was cooled, was diluted to 10 mL with H_2O, and was washed with CH_2Cl_2 (3 x 5 mL). The aqueous layer was concentrated *in vacuo* and the residue was purified by ion exchange chromatography (AG50 x 8, 5 mL, H^+ form), using H_2O and 2% to 10% aqueous NH_4OH as eluents , to give 8.19 mg (83%) of a colorless powder. Spectral data were the same as for **1**. Purity of the material was determined to be greater than 90% by ^{1}H NMR.

(2*S*, 6*R*)- 2,6-diaminoheptanedioic acid (1a) From 117 generated via Triflate Displacement

The same procedure used to prepare **1b** from **117** was used to prepare **1a** from **118** (24.8 mg, 0.0535 mmol) in 72% yield , using 5 mL 5.7M HCl and heating for 2h. Spectral properties were the same as for **1**. Purity of the material was determined to be greater than 90% by ^{1}H NMR.

2,6-bis-(*N*-carbobenzyloxy)amino-1,7-heptanediamide (104) [324]

The procedures of Bodanszky and Bodanszky[221] were adapted. Dry triethylamine (30.0 mL, 2.15 mmol) was added to a cooled (0 °C) solution of D,D + L,L-**19b** (4.70 g, 10.3 mmol) in dry THF (200 mL) and the mixture was stirred for 15 min. Ethyl chloroformate (20.5 mL, 20.9 mmol) was added over 5 min to the resultant slurry, and the reaction mixture was stirred for 30 min. NH_4OH (50 mL) was added over 3 min and the thick slurry was stirred a further 45 min. The mixture was filtered and the precipitate was washed with 10% NH_4OH / water (100 mL). Drying of the solid at room temperature gave

3.71 g (79%) of a colorless powder: mp 224.5-226 °C (lit.[86] mp 223-224 °C); IR (KBr disk) 3385, 3315, 1683, 1657, 1546, 1256 cm^{-1}; ^1H NMR (200 MHz, d_6 DMSO) δ 7.56-7.21 (m, 12H, ArH, NH), 7.00 (br s, 4H, 2 NH_2), 5.04 (s, 4H, CH_2Ar), 4.02-3.80 (m, 2H, 2 CHNH), 1.75-1.22 (m, 6H, $CH_2CH_2CH_2$); MS (Pos FAB, glycerol) 457.19 (457.20 calcd for MH^+). Anal. Calcd for $C_{23}H_{29}N_4O_6 \cdot 1/2\ H_2O$: C, 59.34; H, 6.49; N, 12.04. Found: C, 59.25; H, 6.23; N, 12.30.

2,6-Diaminoheptanediamide Diacetate (105)[324]

The procedure of Arnold[324] was followed. Pd on carbon (5%, 0.283 g) was added to a suspension of **104** (7.00 g, 15.3 mmol) in AcOH (130 mL). The flask was flushed with H_2 and stirred under H_2 (1 atm) for 12 h. The mixture was filtered through Celite, the Celite was washed with AcOH, and the filtrate was concentrated in vacuo to give 5.23 g (99%) of the diacetate salt as a dihydrate: IR (KBr disk) 3420, 1697, 1630, 1614, 1562 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.32-4.20 (m, 2H, 2 CHNH), 2.32-1.80 (m, 4H, 2 CH_2) 2.10 (s, 6H, CH_3CO_2), 1.82-1.50 (m, 2H, CH_2); MS (Pos FAB, glycerol HCO_2H) 189.10 (189.13 calcd for $C_7H_{16}N_4O_2$ + 1). Purity of the material was determined to be greater than 92% by ^1H NMR.

Dimethyl (2*S*, 6*S*)-2,6-Bis([(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-(carbonylamino)heptanedioate (107)

The procedure of Arnold was adapted. LL-DAP (**1b**) (1.93 mg, 0.0102 mmol) was dissolved in 1 M $NaHCO_3$ / Na_2CO_3 buffer, pH 10.4 (2 mL). Toluene (1 mL) was added followed by (1*S*,4*R*)-(-)- camphanic chloride (10.2 mg, 0.0469 mmol) and the mixture was stirred vigorously at room temperature for 2.5 hours. The solution was acidified to pH 1 with 5.7M HCl. It was then diluted to 5 mL with water and extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried over Na_2SO_4 and were concentrated *in vacuo* . The residue was taken up in ether (2 mL). This was

treated with a solution of CH_2N_2 in ether until a yellow color persisted. The ether was evaporated to give 17.1 mg of a waxy solid, which was heated at 65-70 °C for 5 hours at 0.4 mm Hg to give 5.16 mg (88%) of a colorless oil: $[\alpha]_D$= -4.7 ° (c=1.3, $CHCl_3$); IR ($CHCl_3$ cast) 3416, 3365, 2965, 1793, 1745, 1674, 1527 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (br d, *J*= 8.2 Hz, 2H, 2 NH), 4.63-4.57 (m, 2H, 2 CHNH), 3.75 (s, 6H, OCH_3), 2.53-2.42 (m, 2H, 2 CH_aHCR_2CON), 1.99-1.82 (m, 6H, 2 CH_aHCR_2CON, 2 $CH_aHCR_2CO_2$, 2 CH_aHCHN), 1.78-1.47 (m, 4H, 2 $CHH_bCR_2CO_2$, 2 CHH_bCHN), 1.46-1.32 (m, 2H, CH_2), 1.12 (s, 6H, 2 CH_3), 1.11 (s, 6H, 2 CH_3), 0.92 (s, 6H, 2 CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.9 (CO_2R), 171.6 (CO_2R), 167.1 (CONH), 92.2 (NCO), 77.2 (CHNH), 55.3 (OCH_3), 53.9 (CR_4), 52.6 (CR_4), 51.6 (CH_3), 32.0 (CH_2), 31.8 (CH_2), 30.4 (CH_2), 29.1 (CH_2), 21.5 (CH_2), 16.7 (CH_3), 16.6 (CH_3), 9.7 (CH_2); exact mass 578.2824 (578.2839 calcd for $C_{29}H_{42}N_2O_{10}$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dimethyl (2*R*, 6*R*)-2,6-Bis([(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-(carbonylamino)heptanedioate (108)

Employing the same procedure as for **107**, **108** was prepared from D,D-DAP (**1c**) (0.86 mg, 0.0045 mmol) in 85% yield, using (*S*)-camphanic acid chloride (98%, 14.0 mg 6.33 mmol), buffer (2mL) and toluene (2 mL): $[\alpha]_D$= -13.5 ° (c=0.11, $CHCl_3$); IR ($CHCl_3$ cast) 3365, 2965, 1790, 1745, 1672, 1528 cm^{-1} ; ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (br d, *J*= 8.3 Hz, 2H, 2 NH), 4.63-4.57 (m, 2H, 2 CHNH), 3.75 (s, 6H, OCH_3), 2.64-2.50 (m, 2H, 2 CH_aHCR_2CON), 2.02-1.85 (m, 6H, 2 CH_aHCR_2CON, 2 $CH_aHCR_2CO_2$, 2 CH_aHCHN), 1.85-1.62 (m, 4H, 2 $CHH_bCR_2CO_2$, 2 CHH_bCHN), 1.50-1.38 (m, 2H, CH_2), 1.12 (s, 6H, 2 CH_3), 1.08 (s, 6H, 2 CH_3), 0.96 (s, 6H, 2 CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.1 (CO_2R), 171.8 (CO_2R), 167.2 (CONH), 92.3 (NCO), 55.4 (OCH_3, CHNH)), 54.2 (CR_4), 52.5 (CR_4), 51.4 (CH_3), 31.5 (CH_2), 30.4 (CH_2), 29.1 (CH_2), 21.7 (CH_2), 16.7 (CH_3), 16.6 (CH_3), 9.7 (CH_2); exact mass 578.2836

(578.2839 calcd for $C_{29}H_{42}N_2O_{10}$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(2*R*, 6*S*)-2,6-Bis([(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-(carbonylamino)heptanedioate (109)

Employing the same procedure as for **107**, **109** was prepared from *meso*- DAP (**1a**) (32.2 mg, 0.169 mmol), using (S)- camphanic acid chloride (98%, 152 mg, 0.685 mmol) in buffer (3 mL), and toluene (3 mL). The remaining traces of **16** were removed by flash chromatography (30% EtOAc / hexanes, then EtOAc) to give **109** in 87% yield as an amorphous solid after trituration with hexanes: IR ($CHCl_3$ cast) 3360, 2966, 1792, 1746, 1674, 1528 cm^{-1} ; ^1H NMR (360 MHz, $CDCl_3$) δ 6.92 (br d, *J*= 8.3 Hz, 1H, NH), 6.80 (br d, *J*= 8.0 Hz, 1H, NH), 4.65-4.51 (m, 2H, 2 CHNH), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.58-2.40 (m, 2H, 2 CH_aHCR_2CON), 2.05-1.82 (m, 6H, 2 CH_aHCR_2CON, 2 $CH_aHCR_2CO_2$, 2 CH_aHCHN), 1.80-1.62 (m, 4H, 2 $CHH_bCR_2CO_2$, 2 CHH_bCHN), 1.45-1.30 (m, 2H, CH_2), 1.08 (m, 9H, 3 CH_3), 1.03 (s, 3H, CH_3) 0.93 (s, 3H, $2CH_3$), 0.90 (s, 3H, $2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.0 (CO_2R), 177.8 (CO_2R), 171.62 (CO_2R), 171.55 (CO_2R), 167.0 (CONH), 92.21 (NCO), 92.15 (NCO), 55.3 (CHNH), 55.2 (OCH_3), 54.1 (CR_4), 53.9 (CR_4), 52.5 (CR_4), 52.4 (CR_4), 51.53 (CH_3), 51.47 (CH_3), 51.4 (CH_3), 51.3 (CH_3), 31.9 (CH_2), 31.8 (CH_2), 31.4 (CH_2), 30.3 (CH_2), 29.0 (CH_2), 21.5 (CH_2), 16.6 (CH_3), 16.5 (CH_3), 16.4 (CH_3), 9.6 (CH_2); exact mass 578.2832 (578.2839 calcd for $C_{29}H_{42}N_2O_{10}$). Anal. Calcd for $C_{29}H_{42}N_2O_{10}$: C, 60.19; H, 7.32; N, 4.84. Found: C, 60.03; H, 7.59; N, 4.56.

Dimethyl (2*RS*, 6*RS*)-2,6-Bis([(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-(carbonylamino)heptanedioate (110)

Employing the same procedure as for **107**, **110** was prepared from commercial DAP (**1**) (mixture of all possible isomers) (47.3 mg, 0.249 mmol), using (*S*)-camphanic acid chloride (98%, 226 mg, 1.02 mmol), in buffer (3 mL0 and toluene (3 mL). To remove the last traces of methyl camphanoate (**16**), **110** was purified by flash chromatography (30% EtOAc / hexanes, then EtOAc), which after trituration with hexanes gave 0.142 g (99%) of an amorphous solid : IR ($CHCl_3$ cast) 3362, 2966, 1792, 1746, 1674, 1528 cm^{-1} ; ^1H NMR (360 MHz, $CDCl_3$) δ 6.96-6.88 (overlapping br d, *J*= 8.2 Hz, 1H, NH), 4.65-4.54 (m, 2H, 2 CHNH), 3.76 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 2.58-2.42 (m, 2H, 2 CH_aHCR_2CON), 2.05-1.82 (m, 6H, 2 CH_aHCR_2CON, 2 $CH_aHCR_2CO_2$, 2 CH_aHCHN), 1.80-1.62 (m, 4H, 2 $CHH_bCR_2CO_2$, 2 CHH_bCHN), 1.46-1.32 (m, 2H, CH_2), 1.11 (s, 3H, CH_3), 1.08 (s, 3H, CH_3) 0.95 (s, 3H, CH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.1 (CO_2R), 178.0 (CO_2R), 177.9 (CO_2R), 177.8 (CO_2R), 171.72 (CO_2R), 171.66 (CO_2R), 171.6 (CO_2R), 171.5 (CO_2R), 167.2 (CONH), 167.0 (CONH), 92.3 (NCO), 92.25 (NCO), 92.2 (NCO), 92.15 (NCO), 55.3 (CHNH), 55.2 (OCH_3), 54.1 (CR_4), 53.9 (CR_4), 52.5 (CR_4), 52.4 (CR_4), 51.6 (CH_2), 51.5 (CH_2), 51.47 (CH_2), 51.4 (CH_2),32.0 (CH_2),31.9 (CH_2), 31.4 (CH_2), 30.3 (CH_2), 29.0 (CH_2), 21.6 (CH_2), 21.55 (CH_2), 21.5 (CH_2), 16.7 (CH_3), 16.6 (CH_3), 16.4 (CH_3), 9.6 (CH_3); exact mass 578.2839 (578.2839 calcd for $C_{29}H_{42}N_2O_{10}$). Anal. Calcd for $C_{29}H_{42}N_2O_{10}$: C, 60.19; H, 7.32; N, 4.84. Found: C, 60.47; H, 7.37; N, 4.59.

1-Methyl 7-[(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-hydroxyheptanedioate (111)

The procedure of Sakaitani *et al.*[339] was followed. To a solution of **91** (2.68 g, 5.41 mmol) and di-*tert* -butyl pyrocarbonate (1.56 g, 7.06 mmol) in MeOH (20 mL) was added

5% Pd on carbon (0.0479 g) in MeOH (5 mL). The suspension was stirred under H_2 (1 atm) for 20 h and then filtered through Celite. The Celite was washed with MeOH and the combined filtrates were concentrated *in vacuo* to give a colorless oil. Purification by flash chromatography ($CHCl_3$ followed by 3% MeOH / $CHCl_3$ 97%) afforded 2.10 g (84% yield) of a colorless oil: $[\alpha]_D$ = -8.3 ° (c= 0.58, $CHCl_3$); IR (CH_3OH cast) 3480 br, 3410 br, 2935, 2865, 2858, 1742, 1716, cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.36-7.12 (m, 5H, ArH), 5.03 (ddd, 1H, *J*= 11.0, 10.4, 4.3 Hz, CHOR), 4.87 (br d, 1H, *J*= 7.9 Hz, NH), 4.12 (m, 1H, CHNH), 3.94 (m, 1H, CHOH), 3.73 (s, 3H, OCH_3), 2.74-2.60 (m, 2H, CHAr, OH), 2.24-2.18 (m, 1H, CH_aHCHOR), 2.01-1.70 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.66-1.05 (m, 8H, 4 CH_2), 1.46 (s, 9H, $C(CH_3)_3$), 1.00-0.78 (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3 (CO_2R), 173.1 (CO_2R), 155.2 (CONH), 142.8 (Ar), 128.2 (Ar), 127.4 (Ar), 127.3 (Ar), 126.6 (Ar), 126.5 (Ar), 79.4 ($C(CH_3)_3$) 77.7 (CHOR), 69.8 (CHOH), 53.1 (CHNH), 52.0 (OCH_3), 49.8 (CHAr), 33.8 (CH_2), 33.3 (CH_2), 32.1 (CH_2), 32.0 (CH_2), 28.2 (CH_3), 25.5 (CH_2), 24.5 (CH_2), 20.3 (CH_2); exact mass 463.2547 (463.2570 calcd for $C_{25}H_{37}NO_7$). Anal. Calcd. for $C_{25}H_{37}NO_7$: C, 64.78; H, 8.05; N, 3.02. Found C, 64.55; H, 7.69; N, 2.94.

1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-hydroxyheptanedioate (112)

Alcohol **112** was prepared in 80% yield from **92** (0.512 g, 1.03 mmol) by the same procedure used for the synthesis of **111**, employing Boc-anhydride (99%, 0.291 g, 1.32 mmol) and 5% Pd on carbon (0.109 g) in 11 mL MeOH: $[\alpha]_D$= +17.7 (c=1.3 , $CHCl_3$); IR ($CHCl_3$ cast) 3380, 2936, 1737, 1719, 1253, 1210, 1165, 1113 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.34-7.16 (m, 5H, ArH), 5.10 (ddd, 1H, *J*= 11.0, 10.4, 4.3 Hz, CHOR), 4.85 (d, 1H, *J*=8.6 Hz, NH), 4.18-4.08 (m, 1H, CHNH), 3.99-3.90 (m, 1H, CHOH), 3.74 (s, 3H, OCH_3), 2.74-2.64 (m, 1H, CHAr), 2.62 (d, 1H, *J*= 5.5 Hz, OH).

2.22-2.10 (m, 1H, CH_aHCHOR), 2.00-1.87 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.66-0.70 (m, 10H, 5 CH_2), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR δ 174.2 (CO_2R), 173.0 (CO_2R), 155.2 (CONH), 142.6 (Ar), 128.2 (Ar), 128.1 (Ar), 127.2 (Ar), 126.64 (Ar), 126.58 (Ar), 79.5 ($C(CH_3)_3$), 77.6 (CHOR), 69.6 (CHOH), 53.0 (CHNH), 51.9 (OCH_3), 49.7 (CHAr), 33.6 (CH_2), 33.1 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 28.1 (CH_3), 25.5 (CH_2), 24.5 (CH_2), 19.7 (CH_2); exact mass 404.2426 (M^+-$C_2H_3O_2$). Anal. Calcd. for $C_{25}H_{37}NO_7$: C,64.78; H, 8.05; N, 3.02. Found C, 64.83; H, 7.75; N, 2.83.

1-Methyl 7-(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl (2*S*, 6*R*) -2-(*N*-*tert*-Butoxycarbonyl)amino-6-[(methylsulfonyl)oxy]heptanedioate (113)

The method of Girodeau *et al.*[60] was adapted. Methanesulfonyl chloride (98% 0.220 mL, 2.75 mmol) was added to a stirred solution of **111** (0.965 g, 2.08 mmol) in dry pyridine (20 mL) under argon. The mixture was stirred for 1.75 h and was then quenched by the addition of 20% $CuSO_4$·5 H_2O solution (30 mL). The solution was extracted with CH_2Cl_2 (3 x 50 mL), and the combined organic phases were washed with 2M HCl (3 x 30 mL), dried over Na_2SO_4, and then concentrated *in vacuo* to yield 1.05 g (94%) of an oil: $[\alpha]_D$= +14.00 ° (c= 1.4, $CHCl_3$); IR ($CHCl_3$ cast) 3380, 2935, 2860, 1746, 1714, 1365, 1208, 1176 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.36-7.17 (m, 5H, ArH), 5.06 (ddd, 1H, *J*=11.0, 10.4, 4.3 Hz, CHOR), 4.90 (br d, 1H, *J*=8.6 Hz, NH), 4.76 (dd, 1H, *J*= 6.1, 5.5 Hz, $CHOSO_2$), 4.22-4.12 (m, 1H, CHNH), 3.75 (s, 3H, OCH_3), 2.95 (s, 3H, OSO_2CH_3), 2.77-2.66 (m, 1H, CHAr), 2.23-2.15 (m, 1H, CH_aHCHOR), 2.03-1.78 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.70-0.90 (m, 10H, 5 CH_2), 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 172.9 (CO_2R), 168.1 (CO_2R), 155.2 (CONH), 142.8 (Ar), 128.5 (Ar), 128.4 (Ar), 127.6 (Ar), 127.4 (Ar), 126.7 (Ar), 79.8 ($C(CH_3)_3$), 78.1 (CHOR), 77.3 ($CHOSO_2$), 52.9 (CHNH), 52.2 (OCH_3), 49.8 (CHAr), 38.7 (CH_3SO_2), 34.1 (CH_2), 32.1 (CH_2), 31.8 (CH_2), 31.0 (CH_2), 28.2 (CH_3), 25.5 (CH_2), 24.6 (CH_2), 20.3 (CH_2); exact mass 482.2206 (482.2206 calcd for M^+-$C_2H_3O_2$), MS (Pos

FAB, Cleland) 542.53 (542.23 calcd for MH^+). Anal. Calcd for $C_{26}H_{39}NO_9S$: C, 57.65; H, 7.26; N, 2.58; S, 5.92. Found: C, 57.53; H, 7.15; N, 2.70; S, 5.66.

1-Methyl 7-(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl (2*S*, 6*S*) -2-(*N*-*tert*-Butoxycarbonyl)amino-6-[(methylsulfonyl)oxy]heptanedioate (114)

Mesylate **114** was prepared from **112** (1.47 g, 3.23 mmol) in 94% yield in the same manner as **113** was prepared from **111**, using methanesulfonyl chloride (98%, 0.330 mL, 4.18 mmol), in dry pyridine (20 mL): $[\alpha]_D$= +2.9 ° (c= 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3380, 2936, 1749, 1715, 1365, 1210, 1176 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.36-7.18 (m, 5H, ArH), 5.06 (ddd, 1H, *J*=11.0, 10.4, 4.3 Hz, CHOR), 4.90 (br d, 1H, *J*=8.0 Hz, NH), 4.82-4.76 (m, 1H, $CHOSO_2$), 4.22-4.14 (m, 1H, CHNH), 3.74 (s, 3H, OCH_3), 2.95 (s, 3H, OSO_2CH_3), 2.77-2.66 (m, 1H, CHAr), 2.23-2.15 (m, 1H, CH_aHCHOR), 2.03-1.78 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.70-0.90 (m, 10H, 5 CH_2), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 172.9 (CO_2R), 168.1 (CO_2R), 155.2 (CONH), 142.8 (Ar), 128.4 (Ar), 127.3 (Ar), 126.7 (Ar), 79.7 ($C(CH_3)_3$), 78.1 (CHOR), 77.8 ($CHOSO_2$), 52.9 (CHNH), 52.1 (OCH_3), 49.7 (CHAr), 38.6 (CH_3SO_2), 34.0 (CH_2), 32.0 (CH_2), 31.4 (CH_2), 30.8 (CH_2), 28.1 (CH_3), 25.5 (CH_2), 24.5 (CH_2), 19.9 (CH_2); exact mass 482.2206 (482.2206 calcd for M^+-$C_2H_3O_2$), MS (Pos FAB, Cleland) 542.27 (542.23 calcd for MH^+). Anal. Calcd for $C_{26}H_{39}NO_9S$: C, 57.65; H, 7.26; N, 2.58; S, 5.92. Found: C, 57.82; H, 7.25; N, 2.48; S, 5.67.

1-Methyl 7-(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl (2*S*, 6*S*)-2-(*N*-Butoxycarbonyl)amino-6-azidoheptanedioate (115)

Mesylate **113** (0.819 g, 1.51 mmol) and NaN_3 (0.400 g, 6.16 mmol) were dissolved in dry DMF (15 mL) and stirred under argon for 28 h. The DMF was removed *in vacuo* and the residue was taken up in CH_2Cl_2 (75 mL) and washed with brine (2 x 30 mL). The

organic layer was dried over Na_2SO_4 and then concentrated *in vacuo* to give a red oil which was purified by flash chromatography (EtOAc 25% / hexane 75%) to afford 0.647 g (88% yield) of a colorless oil: $[\alpha]_D$= -4.1 ° (c=2.2, $CHCl_3$) ; IR ($CHCl_3$ cast) 3375, 2936, 2861, 2107, 1739, 1717 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.33-7.16 (m, 5H, ArH), 5.06 (ddd, 1H, *J*=11.0, 10.4, 4.3 Hz, CHOR), 4.94 (br d, 1H, *J*=8.6, NH), 3.73 (s, 3H, OCH_3), 3.47 (dd, 1H, J=7.3, 6.7 Hz, CHN_3), 2.72 (ddd, 1H, *J*=12.2, 11.0, 3.7 Hz, CHAr), 2.16-2.10 (m, 1H, CH_aHCHOR), 2.02-1.73 (m, 3H, CHH_bCHO, CH_2CHAr), 1.72-1.02 (m, 10H, 5 CH_2), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (MHz, $CDCl_3$) δ 172.9 (CO_2R), 169.3 (CO_2R), 155.2 (CONH),142.5 (Ar), 128.4 (Ar), 127.5 (Ar), 126.7 (Ar), 79.8 ($C(CH_3)_3$), 77.7 (CHOR), 61.7 (CHN_3), 52.9 (CHNH), 52.2 (OCH_3), 49.9 (CHAr), 33.8 (CH_2), 32.1 (CH_2), 31.8 (CH_2), 30.5 (CH_2), 28.2 (CH_3), 25.6 (CH_2), 24.7 (CH_2), 21.3 (CH_2); MS (Pos FAB Glycerol) 463.36 (463.27 calcd for MH^+). Anal. Calcd for $C_{25}H_{36}N_4O_6$: C, 61.46; H, 7.43; N, 11.47. Found: C, 61.65; H, 7.70; N, 11.40.

Alternative Method to Prepare 1-Methyl 7-[(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-azidoheptanedioate (115)

The procedure of Feenstra *et al.*[259] was used. Triflic anhydride (0.150 mL, 1.15 mmol) was added to a cooled (-78 °C) solution of **111** in CH_2Cl_2 (5 mL), and the solution was stirred for 5 min. 2,6 Lutidine (0.120 mL, 1.04 mmol) was then added to the solution and the mixture was stirred for 10 min. Tetraethylammonium azide (0.216 g, 1.25 mmol) was added and the solution stirred for a further 45 min. The CH_2Cl_2 was removed *in vacuo* and the residue was purified by flash chromatography (33% EtOAc / 67% hexanes) to afford 0.2154 g (72%) of an oil: spectral properties were the same those for **115** prepared in previous method.

1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-azidoheptanedioate (116)

Azide **116** was prepared from **114** (1.12 g, 2.06 mmol) in 77% yield by the same method as employed for the preparation of **115** from **113**, using NaN_3 (0.552 g, 8.49 mmol) in dry DMF (20 mL): $[\alpha]_D$= +25.3 ° (c=1.4, $CHCl_3$) ; IR ($CHCl_3$ cast) 3375, 2936, 2861, 2107, 1739, 1717 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.38-7.16 (m, 5H, ArH), 5.06 (ddd, 1H, *J*=11.0, 10.4, 4.3 Hz, CHOR), 4.94 (br d, 1H, *J*=8.6, NH), 3.74 (s, 3H, OCH_3), 3.45 (dd, 1H, J=7.9, 6.7 Hz, CHN_3), 2.72 (ddd, 1H, *J*=11.0, 11.0, 3.7 Hz, CHAr), 2.22-2.12 (m, 1H, CH_aHCHOR), 2.04-1.78 (m, 3H, CHH_bCHO, CH_2CHAr), 1.78-1.06 (m, 10H, 5 CH_2), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (MHz, $CDCl_3$) δ 172.9 (CO_2R), 169.3 (CO_2R), 155.2 (CONH),142.5 (Ar), 128.3 (Ar), 127.5 (Ar), 126.7 (Ar), 79.9 ($C(CH_3)_3$), 77.7 (CHOR), 61.7 (CHN_3), 53.0 (CHNH), 52.2 (OCH_3), 49.8 (CHAr), 33.7 (CH_2), 32.1 (CH_2), 31.9 (CH_2), 30.5 (CH_2), 28.2 (CH_3), 25.6 (CH_2), 24.7 (CH_2), 21.3 (CH_2); MS (Pos FAB Glycerol) 463.36 (463.27 calcd for MH^+). Anal. Calcd for $C_{25}H_{36}N_4O_6$: C, 61.46; H, 7.43; N, 11.47. Found: C, 61.28; H, 7.62; N, 11.50.

Alternative Procedure for 1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-azidoheptanedioate (116)

The same method employed in the preparation of **115** from **111** was used to generate **116** from **112** (0.191 g, 0.412 mmol) in 76% yield. Triflic anhydride (0.105 mL, 0.807 mmol), 2,6 lutidine (0.080 mL, 0,691 mmol), and tetraethylammonium azide (0.225 g, 1.31 mmol) and **112** were used, and the mixture stirred for 1.5 h: spectral properties were the same those for **116** prepared in previous method.

1-Methyl 7-[(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-aminoheptanedioate (117)

Azide **115** (0.645 g, 1.32 mmol) in MeOH (10.0 mL) was added to 5% Pd on carbon (0.0805 g) moistened with MeOH. The mixture was then stirred under H_2 (1 atm) for 1.5 h. It was filtered through Celite, and the Celite was washed with MeOH. The combined filtrates were concentrated *in vacuo* to give 0.485 g (80% yield) of an oil: $[\alpha]_D$= -4.7 ° (c=0.87, $CHCl_3$); IR ($CHCl_3$ cast) 3370, 2934, 1743, 1716, 1166 cm^{-1}; ^1H HMR (360 MHz $CDCl_3$) δ 7.38-7.12 (m, 5H, ArH), 5.16-4.89 (m, 2H, CHOR, $CHNH_2$), 4.25-4.08 (m, 1H, CHNH), 3.73 (s, 3H, OCH_3), 2.73-2.59 (m, 1H, CHAr), 2.18-0.80 (m, 16H, 7 CH_2, NH_2), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.2 (CO_2R), 172.8 (CO_2R), 155.0 (CONH), 142.5 (Ar), 127.9 (Ar), 127.1 (Ar), 126.1 (Ar), 76.0 (CHOR), 53.7 ($CHNH_2$), 52.8 (CHNH), 51.6 (OCH_3), 49.4 (CHAr), 33.2 (CH_2), 31.8 (CH_2), 31.6 (CH_2), 27.9 (CH_3), 25.3 (CH_2), 24.3 (CH_2), 20.9 (CH_2); exact mass 462.2734 (462.2730 calcd for $C_{25}H_{38}N_2O_6$). Purity of the material was determined to be greater than 95% by ^1H NMR.

1-Methyl 7-[(1*S*, 2*R*)-*trans*-2-Phenylcyclohexyl] (2*S*, 6*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-aminoheptanedioate (118)

Amine **118** was prepared from **116** (0.775 g, 1.59) in 93% yield in a manner analogous to the synthesis of **117** from **115**, using 5% Pd on carbon (0.489 g) in MeOH (15 mL): $[\alpha]_D$= +23.1 ° (c=1.2, $CHCl_3$); IR ($CHCl_3$ cast) 3370, 2973, 2931, 2858,1741, 1714, 1165 cm^{-1}; ^1H NMR (360 MHz $CDCl_3$) δ 7.36-7.18 (m, 5H, ArH), 5.20-5.00 (m, 2H, CHOR, $CHNH_2$), 4.45-4.10 (m, 3H, CHNH, NH_2), 3.76 (s, 3H, OCH_3), 2.76-2.66 (m, 1H, CHAr), 2.23-1.00 (m, 14H, 7 CH_2), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3 (CO_2R), 172.8 (CO_2R), 155.0 (CONH), 142.5 (Ar), 127.9 (Ar), 127.2 (Ar), 126.2 (Ar), 79.2 ($C(CH_3)_3$), 76.0 (CHOR), 53.7 ($CHNH_2$), 52.8 (CHNH), 51.6 (OCH_3), 49.5 (CHAr), 33.2 (CH_2), 31.8 (CH_2), 31.6 (CH_2), 27.9 (CH_3), 25.3

(CH_2), 24.3 (CH_2), 20.9 (CH_2); exact mass 462.2731 (462.2730 calcd for $C_{25}H_{38}N_2O_6$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(2*S*, 6*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-aminoheptanedioic Acid (119) The procedure of Evans and coworkers[367] was followed. $LiOH \cdot H_2O$ (0.0999 g, 2.38 mmol) was added to a stirred solution of **117** (0.275 g, 0.595 mmol) in 3:1 THF/ H_2O (20.0 mL) and the solution was stirred at room temperature for 4.5 h. The THF was removed *in vacuo* and the aqueous solution was washed with CH_2Cl_2 (3 x 10 mL). It was then acidified to pH 4 with 5.7 M HCl. Lyophilization yielded a hygroscopic white foam (0.259 g). IR (CH_3OH cast) 3360, 3040, 1687, 1601 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 4.08-3.83 (m, 1H, CHNH), 3.78-3.55 (m, 1H, $CHNH_2$), 2.10-1.12 (m, 6H, 3 CH_2) 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, CD_3OD) δ 179.4 (CO_2R), 174.8 (CO_2R), 157.8 (CONH), 80.4 ($C(CH_3)_3$), 56.7 (CHNH), 55.9 ($CHNH_2$), 33.6 (CH_2), 31.9 (CH_2), 28.8 (CH_3), 22.4 (CH_2); MS (Pos FAB Glycerol/ HCl) 291.02 (291.16 calcd for $C_{11}H_{20}N_2O_6Li_2$), 297.03 (297.18 calcd for $C_{11}H_{20}N_2O_6Li_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

(2*S*, 6*S*)-2-(*N*-tert-Butoxycarbonyl)amino-6-aminoheptanedioic Acid (120) The procedure used for the conversion of **117** to **119** was utilized to prepare **120** (0.216 g) from **118** (0.209 g, 0.452 mmol), employing $LiOH \cdot H_2O$ (0.0765 g, 1.82 mmol) in 3:1 THF/ H_2O (20.0 mL) over 5 h: IR (CH_3OH cast) 3400, 3040, 1686, 1602 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 4.08-3.83 (m, 1H, CHNH), 3.78-3.55 (m, 1H, $CHNH_2$), 2.10-1.12 (m, 6H, 3 CH_2) 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, CD_3OD) δ 179.3 (CO_2R), 174.6 (CO_2R), 157.8 (CONH), 80.2 ($C(CH_3)_3$), 56.8 (CHNH), 56.0 ($CHNH_2$), 33.9 (CH_2), 32.0 (CH_2), 28.8 (CH_3), 22.7 (CH_2); MS (Pos FAB Glycerol/ HCl) 297.03 (297.18 calcd for $C_{11}H_{20}N_2O_6Li_3$). Purity of the material was determined to be greater than 90% by ^1H NMR.

Methyl 2-Hydroxypropanoate (121)

CH_2N_2 in ether was added to a solution of 85% DL-lactic acid (2.03 g, 19.2 mmol) in ether until a permanent yellow color was obtained. The excess CH_2N_2 was removed by bubbling Ar through the solution. The solvent was removed *in vacuo* to give 1.79 g (90%) of a colorless oil: IR ($CHCl_3$ cast) 3340, 1747, 1130, 1097 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 4.36-4.27 (m, 1H, CHOH), 3.80 (s, 3H, OCH_3), 2.94-2.81 (br d, 1H, *J*= 4.9 Hz, OH), 1.43 (d, 3H, *J*=7.3 Hz, CH_3); exact mass 104.0480 (104.0473 calcd for $C_4H_8O_3$). Purity of the material was determined to be greater than 85% by ^1H NMR.

Methyl 2-[(Methylsulfonyl)oxy]propanoate (122) [368]

The procedure of Girodeau[60] was used. Methanesulfonyl chloride (0.270 mL, 3.49 mmol) was added to a cooled (0 °C) solution of **121** (0.29 g, 2.79 mmol) in dry pyridine (10 mL). The solution was stirred at 4 °C for 17 h. The mixture was poured onto 3M HCl / ice (40mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with 2M HCl (30 mL) and brine (30 mL), and then dried over $MgSO_4$. Concentration *in vacuo* produced 0.368 g (73%) of an oil: IR ($CHCl_3$ cast) 1750, 1358, 1176 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 5.35-4.90 (m, 1H, $CHCH_3$), 3.80 (s, 3H, OCH_3), 3.13 (s, 3H, SO_2CH_3), 1.63 (d, *J*=6.0 Hz, CH_3); MS (CI NH_3) 200 (200 calcd for $M^+ + NH_3$). Purity of the material was determined to be greater than 85% by ^1H NMR.

Furfural Oxime (123) [341]

The method of Goto *et al.*[341] was followed. A solution of NaOH (35.00 g, 875 mmol) in water (130 mL) was added over 1 h to a cooled (0 °C) solution of freshly distilled furfural (82.8 g, 862 mmol) and hydroxylamine hydrochloride (60.0 g, 863 mmol) in water (250 mL). Stirring was continued for an additional 15 min, and the mixture was then warmed to

room temperature and filtered. The precipitate was washed with 300 mL cold water, and then was dried for 3 h. Dissolution in ether, drying the solution over Na_2SO_4, and concentration *in vacuo* to 225 mL, followed by addition of hexanes and subsequent crystallization yielded 68.8g (72%) of white needles: mp 88-89 °C (lit.[341] mp 89-91 °C); IR ($CHCl_3$ cast) 3164, 3081, 2862, 2857, 1646, 1478 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.42 (br s, 1H, HON), 7.52 (s, 1H, CH=N), 7.48 (d, 1H, *J*= 1.2 Hz, ArH), 7.33 (d, 1H, *J*= 3 Hz, ArH), 6.54 (dd, 1H, *J*= 3.0, 1.2 Hz, ArH) exact mass 111.0320 (111.0322 calcd for $C_5H_5NO_2$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl 2-[4-Nitrophenylsulfonyloxy]propanoate (124)

The procedure of Girodeau[60] was modified. Dry pyridine (1.40 mL, 17.3 mmol), and 4-nitrophenylsulfonyl chloride (1.84 g, 7.47 mmol) were added to **121** (0.61 g, 5.86 mmol) in dry CH_2Cl_2. The mixture was stirred at room temperature for 3 h, and was then washed with 20% $CuSO_4 \cdot 5\ H_2O$ (20 mL), water (20 mL), saturated $NaHCO_3$ (20 mL), and water (20 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give 0.944 g (56%) of an oil: IR ($CHCl_3$ cast) 1753, 1537, 1378, 1351, 1236, 1207 1189, 1177, 1082 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.49 (d, 1H, *J*= 9.2 Hz, ArH), 8.41 (d, 1H, *J*= 9.2 Hz, ArH), 8.27 (d, 1H, *J*= 9.2 Hz, ArH), 8.16 (d, 1H, *J*= 9.2 Hz, ArH), 5.26-5.04 (m, 1H, $CHCH_3$), 3.71 (s, 3H, CH_3), 1.60 (d, 3H, *J*= 7.3 Hz, CH_3); exact mass 289.0253 (289.0256 calcd for $C_{10}H_{11}NO_7S$). Purity of the material was determined to be greater than 85% by ^1H NMR.

Tetramethylammonium Azide (125)[344]

The procedures of Gutmann *et al.*[344] and Bryant and Rosenwasser[369] were used. In a well-ventilated fumehood, NaN_3 (10.0 g, 154 mmol) in water (100 mL) (Concentration of azide must be less than 10% to avoid potentially explosive mixtures) was carefully applied

to an ion exchange column (AG50W x 8, H^+ form, 80 mL) and eluted with water, monitoring the pH of the eluent. Eluent containing HN_3 was added to tetramethylammonium hydroxide in MeOH (20%, 27 mL, 51.mmol) until pH 8 was reached. The MeOH was removed *in vacuo* and the water was removed by lyophilization. The residue was extracted with CH_3CN (4 x 25 mL) and the extracts were concentrated *in vacuo* to give 0.57 g (11%) of a white solid: IR (KBr disk) 3357, 3292, 3285, 3020, 3013, 2036, 1997, 1631, 1488 cm^{-1}; ^1H NMR (80 MHz, D_2O) δ 3.10 (s, 12H, CH_3); MS (Pos FAB, Cleland) 74.14 (74.10 calcd for $C_4H_{12}N^+$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Tetraethylammonium Azide (126) [369]

Hydrazoic acid solution , generated as in the preparation of **125** from 10.2 g (156 mmol) NaN_3, was added to tetraethylammonium hydroxide in water (20 %, 108.4 mL, 148 mmol) until pH 8 was attained. The water was removed *in vacuo*, and the residue was extracted with acetone (3 x 125 mL). Concentration of the extracts *in vacuo* gave 21.3 g (83%) of a beige solid: IR (KBr disk) 3430, 3359, 2980, 2029, 1634, 1490 cm^{-1}; ^1H NMR (80 MHz, D_2O) δ 3.28 (q, 2H, J= 7.0 Hz, CH_2), 1.45-1.12 (m, 3H, CH_3); MS (Pos FAB, Cleland 130.21 (130.16 calcd for $C_8H_{20}N^+$). Purity of the material was determined to be greater than 95% by ^1H NMR.

1-Methyl 7-(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl (2*S*-,6*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-6-(2-(*N*-*tert*-butoxycarbonyl))hydrazino-1,7-heptanedioate (128)

The procedure of Feenstra *et al.*[259] was followed. Triflic anhydride (0.050 mL, 0.384 mmol) was added to a solution of **111** (0.111g, 0.240 mmol) in CH_2Cl_2 (5 mL) and the solution was stirred for 5 min. 2,6 Lutidine (0.050 mL, 0.432 mmol) was added and the mixture stirred for 10 min. A solution of *t* -butyl carbazate (65.5 mg, 0.495 mmol) in dry

CH_2Cl_2 (5 mL) was added and the solution was stirred a further 45 min. The mixture was washed with 2M HCl (3 x 10 mL) and the organic layer was dried over Na_2SO_4. Concentration *in vacuo* afforded a residue which was purified by flash chromatography (30% EtOAc / 70% hexanes) to give 84.1 mg (61%) of an oil: $[\alpha]_D$= +7.10 ° (c=6.0, $CHCl_3$); IR ($CHCl_3$ cast) 3400, 2938, 1753, 1718, 1418, 1209 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31-7.10 (m, 5H, ArH), 5.05 (ddd, 1H, *J*= 10.6, 10.6, 4.5 Hz, CHOR), 4.92 (br d, 1H, *J*= 8.2 Hz, NH), 4.80 (dd, 1H, *J*= 6.7, 4.7 Hz, CHNHNHR), 4.18-4.10 (m, 1H, CHNH), 3.69 (s, 3H, OCH_3), 2.65 (ddd, 1H, *J*= 11.9, 11.2, 3.5 Hz, CHAr), 2.15-2.03 (m, 1H, CH_aHCHOR), 1.98-1.67 (m, 3H, CHH_bCHOR, CH_2CHAr), 1.67-0.82 (m, 10H, 5 CH_2), 1.43 (s, 18H, 2 $C(CH_3)_3$); ^{13}C NMR (90.5 MHz, $CDCl_3$) δ 172.8 (CO_2R), 166.1 (CO_2R), 155.2 (CONH), 142.5 (Ar), 128.5 (Ar), 127.5 (Ar), 126.9 (Ar), 83.0 (CHNHNHR), 78.9 (CHOR) , 52.7 (CHNH), 52.2 (OCH_3), 49.8 (CHAr), 33.9 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 30.9 (CH_2), 28.2 ($C(CH_3)_3$), 25.5 (CH_2), 24.6 (CH_2), 20.1 (CH_2); exact mass 577.3335 (577.3363 calcd for $C_{30}H_{47}N_3O_8$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dimethyl Dioxirane (129)

The method of Adam and coworkers[370] was followed. OXONE™ (potassium peroxymonosulfate, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (25.0 g, 40.6 mmol) was added to a vigorously stirred solution of H_2O (20 mL), acetone (13 mL, 177 mmol) and $NaHCO_3$ (12.0 g, 143 mmol) under slight vacuum. The distillate collected (9 mL) was titrated in the following way: 1.00 mL of the dimethyl dioxirane solution was added to a 2.00 mL saturated solution of KI. Solid CO_2 was added to remove air from the solution and the mixture kept in the dark for 10 min. H_2O (5.00 mL) was added and aliquots were withdrawn and titrated with 1.59 mM $Na_2S_2O_3$. This analysis showed the solution of dimethyl dioxirane to have a concentration of 0.12M (3% yield).

Methyl (2*S*)-2-Amino-3-phenylpropanoate Hydrochloride (130)

The method of Greenstein and Winitz[360] was followed. L-Phenylalanine (2.06 g, 12.5 mmol) was suspended in dry MeOH (100 mL) and HCl gas was bubbled through for 15 min. The solution was stirred at room temperature for 44 h. The MeOH was removed *in vacuo* and the residue was triturated twice with Et_2O to give 2.68 g (quant.) of a colorless solid: mp 159-160 °C (lit.[371] mp 159-161 °C); $[\alpha]_D$= -3.7 ° (lit.[223] $[\alpha]_D$= -3.6 °); IR (CH_3OH cast) 3000, 1738, 1490 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.40-7.25 (m, 5H, ArH), 4.37 (t, 1H, *J*=6.5 Hz, CHNH), 2.38-2.20 (m, 2H, CH_2); ^{13}C NMR (90.5 MHz, CD_3OD) δ 170.4 (COOR), 135.3 (Ar), 130.4 (Ar), 130.1 (Ar), 128.9 (Ar), 55.2 (CHNH), 53.6 (OCH_3), 37.4 (CH_2); exact mass 179.0943 (179.0946 calcd. for $C_{10}H_{13}NO_2$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Methyl 2-Hydroxyimino-3-phenylpropanoate (131)

The method of Wittman *et al.*[345] was adapted. Compound **130** (86.8 mg, 0.402 mmol) was dissolved in H_2O (5 mL) and sat. $NaHCO_3$ (5 mL) was added. The solution was immediately extracted with $CHCl_3$ (3 x 10 mL), and the combined organic extracts were dried over Na_2SO_4. Concentration *in vacuo* gave a colorless oil which was immediately dissolved in acetone (2 mL) and H_2O (1 mL). The solution was cooled to -42 °C and **129** (7 mL) was added. The mixture was warmed to room temperature and stirred for 1.5 h. The acetone was removed *in vacuo* and the H_2O was removed by lyophilization. The residue was purified by flash chromatography (40% EtOAc / 60% hexanes, 50% EtOAc / 50% hexanes, and EtOAc to give 39.5 mg starting material and 28.3 mg (36%) of a colorless solid: mp 76-77 °C; IR ($CHCl_3$ cast) 3208, 1743, 1733, 1454, 1395, 1218, 1026 1013, 1006 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 10.11 (br s, 1H NOH), 7.33-7.05 m, 5H, ArH), 3.90 (s, 2H, CH_2), 3.72 (s, 3H, CH_3); exact mass 193.0739 (193.0739 calcd

for $C_{10}H_{11}NO_3$). Purity of the material was determined to be greater than 95% by ^1H NMR.

2-Acetamidoheptanedioic Acid (132)

The procedure of Wade *et al.*[86] was adapted. Acetic anhydride (5.00 mL, 52.9 mmol) was added in small portions over 0.5 h to a solution of DL-α-aminopimelate (**14**) (3.52 g, 20.1 mmol) in 2M NaOH (25 mL). NaOH (2M, 20 mL) was added in portions alternately with the acetic anhydride to maintain the pH above pH 10. The mixture was stirred at room temperature for 2 h, occasionally adjusting the pH with 2M NaOH. The mixture was acidified to pH 1, and the solvent was removed *in vacuo*. The residue was dissolved in water (20 mL) and evaporated to dryness *in vacuo*. The residue was then extracted thoroughly with hot acetone and the extracts were concentrated *in vacuo* to 20-30 mL. Ether was added until the solution was slightly turbid. Cooling produced 1.95 g of colorless crystals. Concentration of the mother liquor afforded an additional 2.01 g of crystals for a total of 3.96 g (91%) of a colorless solid: mp 118.5-120.5 °C (lit.[86] mp 111-112 °C); IR (KBr disk) 3319, 1721, 1676, 1565 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.31 (dd, 1H, *J*=9.2, 4.9 Hz, CHNH), 2.41 (t, 2H, *J*= 7.6 Hz, CH_2CO_2H), 2.03 (s, 3H, CH_3)1.95-1.56 (m, 4H, CH_2CH_2), 1.46-1.31 (m, 2H, CH_2); exact mass 217.0956 (217.0950 calcd for $C_9H_{15}NO_5$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Resolution of 2-Acetamidoheptanedioic Acid (132) to (2*R*)-2-Aminoheptanedioic Acid (133) and (2*S*)-2-Aminoheptanedioic Acid (15)

The procedures of Wade *et al.*[86] and Berges *et al.* [56]were used. Acetamide **132** (2.50 g, 11.5 mmol) was dissolved in water (115 mL) and the pH was adjusted to 7.55 with 2M LiOH (12.0 mL) and AcOH (2 drops). Hog kidney acylase I (1.00 g) was added and the mixture was agitated at 39 °C for 4.5 h. The pH of the solution was readjusted to pH 7.5

at 0.25h and 0.75 h after initiation of the reaction. The reaction was quenched by acidification to pH 4.88 and diluted to 500 mL with H_2O. Norit (2.06 g) was added and the solution was filtered. The filtrate was acidified to pH 3.44 and an additional 3.98 g of Norit was added. Filtration and evaporation of the solvent *in vacuo* produced a residue which was dissolved in water and loaded onto an ion exchange column (AG50Wx8, H^+ form, 200 mL). Elution with water (500 mL) gave 0.88 g (70%) of the D-*N*-acetyl compound **134:** mp 119-123 °C; IR (KBr disk) 3345, 2950, 1722, 1673, 1619, 1551, 1268, 1247, 1212 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.31 (dd, 1H, *J*=8.8, 5.2 Hz, CHNH), 2.41 (t, 2H, *J*= 7.3 Hz, CH_2CO_2H), 2.03 (s, 3H, CH_3)1.95-1.56 (m, 4H, CH_2CH_2), 1.46-1.31 (m, 2H, CH_2); exact mass 217.0929 (217.0950 calcd for $C_9H_{15}NO_5$). Purity of the material was determined to be greater than 95% by ^1H NMR.

A 0.47 g portion of **134** was dissolved in 4M HCl (50 mL) and heated to reflux for 2 h. The solution was cooled and concentrated *in vacuo* to give 0.348 g (92%) of amino acid **133**. Recrystallization from water / EtOH yielded 67.3 mg (19%) of colorless crystals: $[\alpha]_D$= -19.8 ° (c=1.0, 5M HCl) (lit.[56] $[\alpha]_D^{26}$= -21.4 °(c=1.0, 5M HCl); IR (KBr disk) 3440, 3070, 1669, 1592, 1508 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.10 (dd, 1H, *J*= 6.3, 6.3 Hz, $CHNH_2$), 2.43 (t, 2H, *J*= 7.2 Hz, CH_2CO_2H), 2.10-1.85 (m, 2H, CH_2), 1.78-1.35 (m, 4H, CH_2CH_2); MS (Pos FAB) (glycerol , HCO_2H) 176.17 (176. 18 calcd for MH^+). Purity of the material was determined to be greater than 90% by ^1H NMR.

Elution of the initial ion exchange column with 1 M NH_4OH (1 L) yielded 0.96 g of the crude L-amino acid **15**. Recrystallization from water / EtOH afforded 0.53 g (53%) of the L-amino acid **15**: $[\alpha]_D$= +20.9 ° (c= 1.1, 5M HCl) (lit.[56] $[\alpha]_D^{26}$= +20.4 °(c=1.0, 5M HCl); IR (KBr disk) 3480, 3080, 1667, 1642, 1508 cm^{-1}; ^1H NMR (200 MHz, D_2O + DCl) δ4.10 (dd, 1H, *J*=6.1, 6.1 Hz, $CHNH_3$), 2.46-2.33 (m, 2H, CH_2CO_2H), 2.08-1.85 (m, 2H, CH_2), 1.75-1.33 (m, 4H, CH_2CH_2), MS (Pos FAB, glycerol / HCO_2H) 176.46 (176.18 calcd for MH^+). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dimethyl (2*S*)-2-([(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-carbonyl]amino)heptanedioate (135)

The procedure of Arnold[324] was adapted. (-)-(1*S*, 4*R*)-Camphanic acid chloride (98%, 21.2 mg, 0.0960 mmol) in toluene (0.50 mL) was added to a solution of **15** (7.24 mg, 0.0.0413 mmol) in 1.0M pH 10 $NaHCO_3$ / Na_2CO_3 buffer (1 mL), and the mixture was stirred vigorously for 2 h. The solution was acidified to pH 1 with 5.7M HCl, diluted to 5 mL with water and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a colorless solid. This residue was dissolved in ether and treated with CH_2N_2 in ether until the yellow color persisted. Concentration *in vacuo* after removal of excess CH_2N_2 afforded a colorless oil. Methyl camphanoate (**16**) was removed by sublimation at 65-70 °C (0.07 mm Hg) for 1.5 h, to leave 9.94 mg (63%) of a colorless oil: $[\alpha]_D$= -23.1 ° (c=1.3, $CHCl_3$); IR ($CHCl_3$ cast) 3385, 2955, 1792, 1740, 1675, 1528, 1124 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.90 (d, 1H, *J*=7.9 Hz, NH), 4.66-4.56 (m, 1H, CHNH), 3.76 (s, 3H, OCH_3), 3.66 (m, 3H, OCH_3) 2.53-2.42 (m, 1H, CH_aHCR_2CON), 2.30 (t, 2H, *J* = 7.6 Hz, CH_2), 2.00-1.81 (m, 3H, CHH_bCCON, $CH_aHCR_2CH_3$, CH_aHCHN), 1.76-1.52 (m, 4H, $CHH_bCR_2CH_3$, CHH_bCHN, CH_2), 1.30-1.26 (m 2H, CH_2), 1.12 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 0.92 (s, 3H, CH_3); exact mass 383.1940 (383.1944 calcd for $C_{19}H_{29}NO_7$). Purity of the material was determined to be greater than 95% by ^1H NMR.

Dimethyl (2*R*)-2-([(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-carbonyl]amino)heptanedioate (136)

Compound **136** was prepared from **133** (2.60 mg, 0.0148 mmol) in 83% yield in an analogous manner to the preparation of **134** from **15**, using (*S*) camphanic acid chloride (98%, 13.5 mg, 0.0623 mmol) in buffer (1 mL), and toluene (0.5 mL): IR ($CHCl_3$ cast) 3390, 2964, 1792, 1739, 1675, 1530, 1265 cm^{-1};^1H NMR (360 MHz, $CDCl_3$) δ 6.78 (d,

1H, *J*=8.6 Hz, NH), 4.59-4.48 (m, 1H, CHNH), 3.75 (s, 3H, OCH_3), 3.67 (m, 3H, OCH_3) 2.53-2.42 (m, 1H, CH_aHCR_2CON), 2.32 (t, 2H, *J* = 7.3 Hz, CH_2), 2.00-1.81 (m, 3H, CHH_bCR_2CON, $CH_aHCR_2CH_3$, CH_aHCHN), 1.80-1.56 (m, 4H, $CHH_bCR_2CH_3$, CHH_bCHN, CH_2), 1.49-1.16 (m, 2H, CH_2), 1.13 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); exact mass 383.1940 (383.1944 calcd for $C_{19}H_{29}NO_7$). Purity of the material was determined to be greater than 90% by ^1H NMR.

Dimethyl (2*SR*)-2-([(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-carbonyl]amino)heptanedioate (137)

Compound **137** was prepared from D,L α-aminopimelate (**14**) (19.6 mg, 0.112 mmol) in 88% yield in an analogous manner to the preparation of **134** from **15**, using (*S*) camphanic acid chloride (98.8 mg, 0.456 mmol) in buffer (2.5 mL), and toluene (0.7 mL): IR ($CHCl_3$ cast) 3368, 2956, 1794, 1739, 1675, 1528, 1265, 1211, 1169 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.90 (d, 1H, *J*=7.9 Hz, NH), 6.80 (d, 1H, *J*=7.9 Hz, NH) 4.67-4.57 (m, 1H, CHNH), 3.76 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.67 (m, 3H, OCH_3), 3.66 (m, 3H, OCH_3), 2.59-2.42 (m, 1H, CH_aHCR_2CON), 2.36-2.25 (m, 2H, CH_2), 2.02-1.81 (m, 3H, CHH_bCR_2CON, $CH_aHCR_2CH_3$, CH_aHCHN), 1.80-1.56 (m, 4H, $CHH_bCR_2CH_3$, CHH_bCHN, CH_2), 1.49-1.28 (m, 2H, CH_2), 1.12 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.92 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.1 (CO_2R), 178.0 (CO_2R), 173.5 (CO_2R), 171.8 (CO_2R), 169.5 (CONH), 166.9 (CONH), 92.2 (CO), 55.2 (CHNH), 53.9 (OCH_3) 52.4 (CR_4), 51.7 (OCH_3), 51.5 (OCH_3), 33.6 (CH_2), 32.0 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 29.0 (CH_2), 24.9 (CH_2), 24.3 (CH_2), 16.7 (CH_3), 16.6 (CH_3), 16.5 (CH_3),9.6 (CH_2); exact mass 383.1946 (383.1944 calcd for $C_{19}H_{29}NO_7$); Anal Calcd for $C_{19}H_{29}NO_7$: C, 59.52; H,7.62; N, 3.65. Found: C, 59.26; H, 7.50; N, 4.05.

Isolation of DAP Epimerase

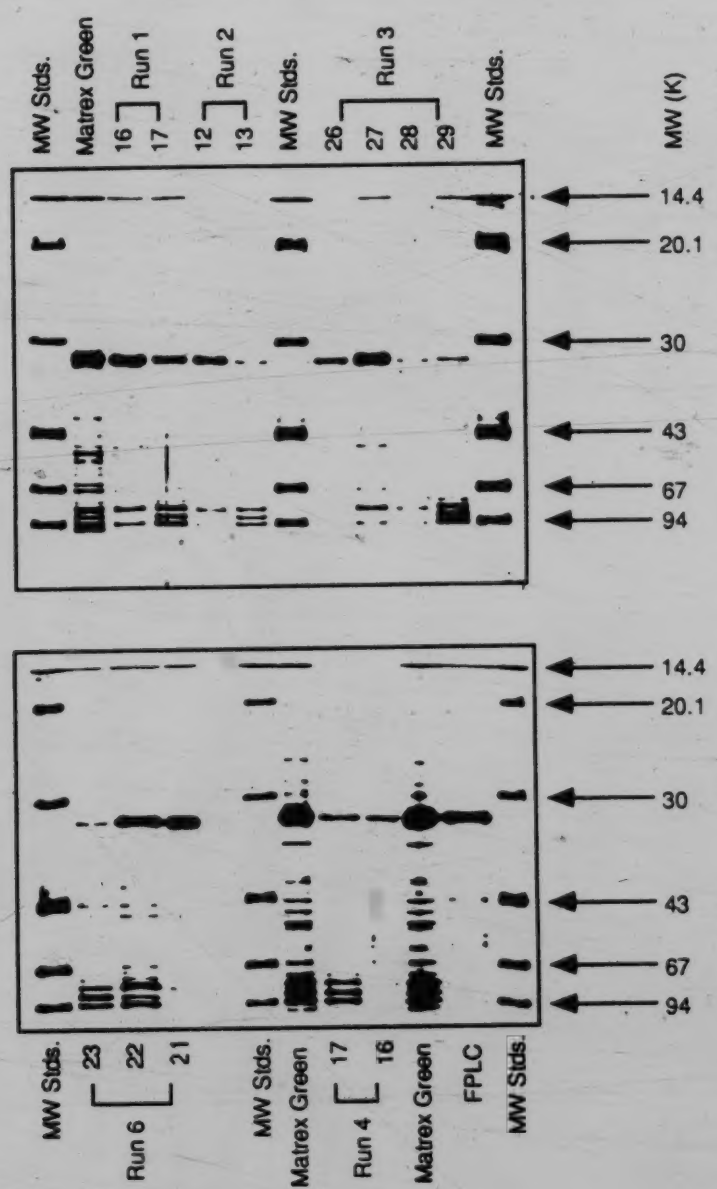
The procedure of Higgins *et al.*[41] was modified. All purification steps were performed at 4 °C. Cells of the strain of *E. coli* JM109, harboring the plasmid pDF6 which contains the epimerase gene (obtained as a generous gift from Dr. William Higgins, Merrell-Dow Research Institute, Strasbourg, France) (354 g wet weight) were washed in buffer A (20mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid (EDTA), 1mM dithiothreitol (DTT) pH 7.0) and resuspended in 1.2 L buffer A. The cells were broken by sonication and the cell debris removed by centrifugation at 16,300 x g for 30 min and 25,400 x g for 1 h, to give 1.6 L of a cloudy brown supernatant. The supernatant was adjusted to 30% saturation with ammonium sulfate ($(NH_4)_2SO_4$), followed by centrifugation at 23,300 x g for 1 h to give a cloudy yellow supernatant and a brown pellet. This supernatant was adjusted to 45% saturation with $(NH_4)_2SO_4$ and centrifuged again under the same conditions to produce a clear yellow supernatant and a brown pellet. The pellets obained from the 30% and 45% $(NH_4)_2SO_4$ fractionations were resuspended in a minimum amount of buffer A and dialyzed 3 times against buffer A, giving a final volume of 225 mL for the 30% pellet and 190 mL for the 45% pellet. The various supernatants and pellets were assayed by the coupled enzyme assay procedure using a mixture of D,D and L,L-DAP as the substrate. Samples of the supernatants obtained during the ammonium sulfate fractionations were devoid of activity by the time they were assayed.

The enzyme solution from the 30% pellet was diluted to 500 mL with buffer A and was adjusted to 15% saturation with $(NH_4)_2SO_4$. Centrifugation (25,400 x g, 45 min) yielded a clear brown supernatant and a brown pellet. The supernatant was adjusted to 45% saturation with $(NH_4)_2SO_4$, was centrifuged as before, and the pellet was resuspended in the minimum amount of buffer A and dialyzed against buffer A to give 60 mL of a brown solution of specific activity 13.6 U/mg. A 20 mL aliquot of this enzyme solution was loaded onto a Matrex Green A affinity column (Amicon)(4.6 cm x 16 cm) and was washed with 750 mL of buffer A. The column was then eluted with a gradient of buffer A

containing 0.0-2.0 M KCl. Active fractions calculated to have similar specific activities were combined and concentrated by ultrafiltration to give 3 enzyme solutions (60 mL each) having specific activities of 34, 36 and 37 U/ mg , respectively.

To prepare the Q-Sepharose column, Q-Sepharose (Pharmacia) was hydrated by suspension in buffer A for 2 days. A Q-Sepharose MPLC column (30 mL) was prepared in an MPLC column with a flow rate of 3 mL / min. Once the column was packed it was equilibrated with 250 mL buffer A. For the initial tests, enzyme solution (5 mL) was loaded on and the column was eluted with buffer A (100 mL), followed by buffer A containing 0.2M NaCl (100mL) and buffer A containing 1M NaCl (110 mL). The column flow rate was 2 mL /min and the pressure was 15-20 psi. Purification was improved by packing the column further and running a linear gradient from 0-0.20M NaCl. In all cases the first active fraction was the purest as determined by specific activity and SDS-PAGE electrophoresis (10% T, 2.7% C , constant current (30 mAmp))(Figure 16).

Figure 16. Electrophoresis Gels of Q-Sepharose Fractions



Runs 1 and 2 - step gradient

Runs 3, 4, 6 - continuous gradient

Assay of DAP Epimerase by Coupled Enzyme Assay

The method of Wiseman and Nichols[40] as modified by Lam *et al.* [55] was followed. DAP epimerase was assayed at 25 °C at pH 7.8 in buffer B (0.10 M Tris·HCl, 1 mM EDTA, 1 mM DTT) containing 0.3 mM NADP in a disposable cuvette with a 1 cm light path by coupling the conversion of L,L-DAP to *meso*-DAP with the NADP-dependent oxidation of *meso*-DAP by *meso*-DAP dehydrogenase. The increase in absorbance was followed at 336-344 nm. This assay is linear over time for a change of up to 0.2 absorbance unit.[40] In this assay 1 unit of epimerase activity is 1 μmol NADPH formed / min under these conditions.

For assays during the isolation of the epimerase, 0.4 mM L,L-DAP or D,D and L,L-DAP (0.4 mM in L,L-DAP) and 18mU of DAP dehydrogenase were present in buffer B and 50 μL aliquots of the fraction in question (diluted if necessary) were added. For the determination of control and inhibition curves for the inhibition studies, concentrations of 0.04 mM, 0.10 mM, 0.20 mM, 0.40mM, 1.0 mM, and 2.0 mM L,L-DAP were used in the presence of 18 mU dehydrogenase and 3.9 mU DAP epimerase. The kinetic constants V_{max} and K_m were obtained from initial rate data using the statistical method of Wilkinson.[346] The K_m determined by this method is 0.24 mM ± 0.02 mM (lit.[55] K_m=0.26 mM ± 0.01 mM).

Inhibition Studies on DAP Epimerase Using the Coupled Enzyme Assay

The assay procedure described above was used with the inclusion of substrate analog (3.0 mM) in the assay mixture. The kinetic constants V_{max} and K_m were obtained from initial rate data using the statistical method of Wilkinson.[346] Competitive inhibition constants K_i were determined using the equation $K_m^{(I)} = K_m(1 + [I] / K_i)$ where $K_m^{(I)}$ and K_m are the apparent K_m values in the presence and absence of inhibitor (I), respectively.[55] Noncompetitive inhibition constants K_i' were determined by the relationship $V_{max} = V_{max}^{(I)} (1 + [I] / K_i')$ where $V_{max}^{(I)}$ and V_{max} are the apparent V_{max} values in the

presence and absence of inhibitor (I), respectively.[55] Results for the mixture of monophosphonates **8** and **9** were as follows:$K_m^{(I)}$ 0.27 mM± 0.06 mM (K_m 0.24 mM ± 0.02 mM), $V_{max}^{(I)}$ 3.6 ± 0.3 x 10^{-2} ΔA/min (where ΔA is the change in absorbance units)(V_{max} 4.6 ± 0.1 x 10^{-2} ΔA/min). For the diphosphonate analog **12** a $K_m^{(I)}$ of 0.41 mM ± 0.09 mM (K_m 0.24 mM ± 0.02 mM), and a$V_{max}^{(I)}$ of 3.8 ± 0.3 x 10^{-2} ΔA/min (V_{max} 4.6 ± 0.1 x 10^{-2} ΔA/min) were determined.

Determination of Protein Content

The method of Bradford[372] was used to determine protein concentration , employing Bio-Rad protein assay dye reagent and the standard assay conditions provided with this reagent. Standard curves were prepared using bovine serum albumin diluted to desired concentrations. The enzyme solution (diluted if necessary) (100 μL) was added to a test tube and 5 mL of dilute Bio-Rad protein assay dye reagent (5 fold dilution) was added. The mixture was vortexed and left to stand for 5 min. The absorbance was then measured at 594-596 nm against a blank solution prepared by the addition of 5 mL dilute dye reagent to 100 μL water.

Assay of DAP Dehydrogenase

For the one point assay, the procedure of Wiseman and Nichols[40] as modified by Lam *et al.*[55] was adapted. *meso*-DAP dehydrogenase activity was assayed at 25 ° C in buffer B containing 0.3 mM NADP, and 1.06 mM *meso*-DAP at pH 7.8 by following the absorbance at 336-344 nm, using a 1cm light path. A unit of activity is 1 μmol / min NADPH formed under these conditions. 1.8 mU of dehydrogenase was used.

For the more detailed control and inhibitor curves in the inhibition studies, the procedure was modified. DAP dehydrogenase (1.8mU) was assayed at 25 °C in buffer B containing 0.3 mM NADP, using *meso*-DAP concentrations of 0.0175 mM, 0.02625 mM, 0.035 mM, 0.070 mM, 0.105 mM, and 0.175 mM (concentrations above 0.175 mM

resulted in substrate inhibition of the enzyme). The K_m determined for *meso*-DAP is 0.092 mM ± 0.008 mM (lit.[40] K_m= 0.11mM).

Assay of Monophosphonate Inhibitors 8-12 as Substrates of DAP Dehydrogenase

The procedure of Lam *et al.*[55] was modified. The inhibitors were tested as substrates of the dehydrogenase by replacing the *meso*-DAP in the standard assay by 2.5 mM inhibitor and monitoring the absorbance at 336-344 nm.

Inhibition Studies of DAP Dehydrogenase with Substrate Analogs

Competitive inhibition studies were performed by following the assay procedure described above, using varying amounts of *meso*-DAP except that 2.25 mM substrate analog was included in the mixture. The kinetic constants V_{max} and K_m were obtained from initial rate data as previously described. Competitive inhibition constants K_i were calculated using the equation $K_m^{(I)} = K_m(1 + [I]/ K_i)$ where $K_m^{(I)}$ and K_m are the apparent K_m values in the presence and absence of inhibitor (I), respectively.[55] Noncompetitive inhibition constants K_i' were determined by the relationship $V_{max} = V_{max}^{(I)} (1 + [I] / K_i')$ where $V_{max}^{(I)}$ and V_{max} are the apparent V_{max} values in the presence and absence of inhibitor (I), respectively.[55] The$V_{max}^{(I)}$ determined for inhibitor **9** was $2.2 \pm 0.2 \times 10^{-2}$ ΔA/min (where ΔA is the change in absorbance units), while V_{max} was found to be $2.6 \pm 0.1 \times 10^{-2}$ ΔA/min.

Assay for Time-dependent Inhibition of DAP Dehydrogenase with Substrate Analogs

The analog (3.0 mM in buffer B) was incubated with the enzyme (180 mU) at 25 °C. Aliquots (10 µl) were removed from this mixture at 0, 30, 60, 90 and 120 min and were added to an assay mixture (0.990 mL) containing 0.175 mM *meso*-DAP, 0.30 mM NADP.

1 mM EDTA, and 1 mM DTT. The activity was monitored as described above. A control mixture with buffer B replacing the inhibitor above was incubated simultaneously for each analog and was assayed in the same manner. Comparison of the initial velocities determined for the control and the inhibitor at each time indicated if there was any time-dependent inhibition.

Radioactive Assay of DAP Epimerase

Preparation of stock solutions: A portion (300 μL) of racemic generally labelled tritiated DAP [G-^{3}H DAP] of specific activity 1.09 mCi/ mmol in H_2O (1 mL) (concentration 1mCi / mL) was evaporated by a gentle stream of Argon in a vial. H_2O (300 μL) was added and the H_2O evaporated in the same manner. This process was repeated twice and the residue remaining was dissolved in H_2O (3.00 mL). This solution (2.31 mL) was added to L,L-DAP (19.3 mg,0.101 mmol) in buffer B (7.84 mL) to give a 10 mM solution of L,L-DAP (22.7 μCi/ mL). This solution was diluted to provide a 1mM DAP solution.

For the radioactive assay, the procedure of Lam *et al.*[55] was used with some modification. The concentrations of L,L-DAP (0.05 mM, 0.10 mM, 0.20 mM, 0.40 mM, 1.0 mM and 2.0 mM) and amount of epimerase (6.0 mU) chosen were based on those used in the coupled enzyme assay. A reaction mixture (200μL) containing DAP epimerase (6 mU) and L,L-DAP in buffer B was incubated for 40 min at 25 °C. The solution was quenched with 10% trichloroacetic acid, vortexed, and applied to a column of ion exchange resin (0.5 mL, Biorad AG 50W X 8, H^+ form, 200-400 mesh). The column was eluted with H_2O (3 x 500 μL) and the eluates were collected and counted in a scintillation counter for 15 min. Each concentration of DAP was run in triplicate with enzyme. A blank and substrate alone were run simultaneously under the same conditions. Kinetic constants K_m and V_{max} were obtained from the nonlinear regression program Enzfitter.(Elsevier Biosoft), using Michaelis-Menten kinetics and explicit weighting. The K_m determined in this manner was 0.31 mM ± 0.03 mM.

One Point Inhibition Test for 4-Fluoro-DAP (18) with the Epimerase

The general procedure decribed above was followed with the following changes: 0.30 mM L,L-DAP was used and 3.0 mM 4-fluoro DAP (**18**) and 3.0 mM *meso*-DAP were each included in the assay mixtures.

Competitive Inhibition Studies of the Epimerase using the Radioactive Assay

The general procedure outlined above was used with the inclusion of substrate analog in the reaction mixture. Kinetic constants K_m and V_{max} were obtained from the nonlinear regression program Enzfitter using Michaelis Menten kinetics and explicit weighting. Competitive inhibition constants K_i were calculated as described earlier.[55] Noncompetitive inhibition constants K_i' were determined as described in reference 55.

Test of Analogs 8 and 9 at Constant Substrate Concentration with DAP Epimerase

The general assay procedure described above was followed using 2 mM L,L-DAP as substrate and including 0, 3.0 mM, 6.0 mM, 9.0 mM, and 15 mM of the inhibitor in the reaction mixture.

Assay for Time-dependent Inhibition of DAP Epimerase with Substrate Analogs

Time-dependent inhibition was tested by incubation of the epimerase (12 mU) in the presence and absence of inhibitor (3.0 mM) at 25 °C. Aliquots (20 μL) were removed at 0, 30, 60, and 120 min and assayed under the conditions outlined above using 0.30 mM L,L-DAP as the substrate.

Test of Analogs as Substrates for DAP Epimerase

Compounds were tested as substrates for the epimerase by incubating 700 mU of active or inactive epimerase in the presence of the inhibitor (3.0 mM, 9 mmol) in deuterated buffer B, pD 7.8 (total volume 3.00 mL) at 25 °C for 6 h. The incubations were quenched by freezing in a dry ice / isopropyl alcohol bath. The mixtures were then thawed and loaded onto ion exchange columns (0.5 mL, Biorad AG1 X 8, OH^- form, 100-200 mesh). The column was washed with water (2 x 1 mL) and eluted with 1M HCl (2.5 mL). The HCl eluents were combined and the H_2O was removed *in vacuo* by a Savant™ Speed Vac concentrator The residue was dissolved in D_2O and the proton NMR spectrum was acquired. The sample was then lyophilized and the residue was dissolved in H_2O, and the H_2O was removed *in vacuo*. This step was repeated twice before the residue was dissolved in H_2O and the deuterium NMR spectrum obtained. In those cases where residual EDTA interfered with the α-H signals in the proton NMR spectrum, these compounds were loaded onto a cellulose column (2.5 cm x 27 cm) equilibrated in *n*-butanol / H_2O / AcOH (4: 2: 1) and eluted with the same solvent. If interference from other impurities was still present, the compound was passed through a second ion exchange column, under the conditions described above, before proton and deuterium NMR spectra were obtained.

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